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Creation of the MALDI-TOF MS Database for Identification of *Loa loa* Microfilaria: Preliminary Study

Elsa-Rush Eyang-Assengone[1,2*], Carole Cassagne[3], Roland Dieki[1,2], Jean Paul Akue[1], Jean Bernard Lekana-Douki[1,4,5], Stéphane Ranque[3,6]

[1]Centre Interdisciplinaire de Recherches Médicales de Franceville (CIRMF), Franceville, Gabon
[2]Unité de Recherches en Ecologie de la Santé (URES), Franceville, Gabon
[3]IHU-Méditerranée Infection, Marseille, France
[4]Unité Ecologie Evolution et Résistances Parasitaires (UNEEREP), Franceville, Gabon
[5]Département de Parasitologie-Mycologie, Université des Sciences de la Santé (USS), Libreville, Gabon
[6]Aix Marseille University, IRD, AP-HM, SSA, Vitrome, Marseille, France
Email: *elsaassengone@gmail.com

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Abstract

Mass spectrometry (MS) is a powerful tool for identifying bacteria and fungi. Several attempts have been made to apply the technique in clinical parasitology but not in filarial identification. The study shows that MALDI-TOF-MS is a promising tool to identify *Loa loa* microfilaria of patients from an endemic country. *Loa loa* microfilariae were isolated and purified from blood via a Percoll gradient. The microfilaria was separated into different groups from 1 microfilaria to 500 microfilaria specimens. Four extraction procedures were subsequently tested to determine the most adaptable procedure for the quality protein for MALDI-TOF MS analysis. A reference database of spectra was constructed and tested on a panel of 80 isolates. A blind test of 80 spectra was performed against the created library. Results showed that the best protocol for protein extraction used an equal volume of 70% formic acid 100% acetonitrile. Good resolution spectra were obtained at 400 microfilariae. The approach correctly identified all the patients. Ten spectra that were used to build a MS. The LSV obtained after comparison of the spectra with the MSP showed a good technical reproducibility ranged from 1.86 to 2.9, with a mean of 2.398 ± 0.046 and a good biological reproducibility with a mean of 2.13 [1.17 - 2.55]. MALDI-TOF MS is a promising approach, providing rapid and accurate identification of *L. loa* microfilaria.

Keywords

Loiasis, Microfilariae, Parasite, Identification, Diagnostic

1. Introduction

Loiasis is endemic in Central and West Africa, where it is estimated that up to 13 million people are infected [1]. *Loa loa* is a nematode transmitted by the infected Chrysops spp. [2] [3]. Adult female worms are viviparous and produce *Loa loa* microfilariae that are usually detectable in the blood [4] [5]. Clinical manifestations include pruritus, subcutaneous swelling (calabar swelling), and migration of the adult parasite (eye worm) [6]. In fact, coinfection between *Loa loa* and *Onchocerca volvulus* (responsible for onchocerciasis) or between *Loa loa* and *Wuchereria bancrofti* and/or *Brugia malayi* (responsible for lymphatic filariasis) may lead to complications such as meningoencephalitis [7] [8]. Complications occur in patients with high *L. loa* microfilarial loads (>30,000 microfilariae/ml of blood) [9] [10]. Therefore, the quantification and differentiation of microfilariae are essential prior to treatment with anthelminthic drugs, whether for individual management or control efforts. In endemic areas, the diagnosis of loiasis relies on microscopic observation of *Loa loa* microfilaria in the blood. But microscopy is associated with limited diagnostic accuracy. For better analysis, serological techniques [11] and molecular methods [12]-[14] have been evaluated for the diagnosis of *L. loa* infection. However, both of these methods are time intensive and relatively expensive (qPCR) [15]. Matrix-assisted laser desorption ionization–time of flight mass spectrometry (MALDI-TOF MS) is based on the detection of specific proteins released from microbial cells [16] [17]. It was demonstrated that different bacterial species exhibit specific protein mass spectra, which can be used for rapid identification [17]. MALDI-TOF MS-based identification of microorganisms involves the generation of mass spectra from whole-cell material or extracted intracellular content, which are subsequently matched to known database references [18]-[20]. An available spectral database containing reference spectra, also called main spectral profiles (MSPs), which are generated from well-characterized specimens, is needed [21] [22]. A key aspect of whole-cell MALDI-TOF MS is the rapid generation and comparison of mass spectra [23]. This analytic technique can analyse the mass-to-charge ratio of numerous biomolecules, such as peptides and proteins [24]. The peaks in these mass spectra represent abundant cellular proteins [21] [25] [26]. In fact, this technique has been used for identifying bacteria [27], fungi [28], and viruses [29] and for entomology [30] [31]. Although the development of MALDI-TOF MS in clinical parasitology has been much more limited compared to that observed in bacteriology or mycology, it is a promising tool for the identification of parasites and has gained popularity because it is a fast, easy-to-use, and cost-effective approach that permits a high-throughput analysis by simultaneous processing of multiple samples [32]. Several attempts have been made to identify parasites [33]-[35]. However, the successful identification of *Trichinella species* by MALDI-TOF MS has been reported by Mayer-Scholl *et al.* [36], after the construction of a reference database with mass spectra from the nine described species of the genus. Recently, for the first time, MALDI-TOF MS was applied for the differentiation of *Entamœba histolytica* and *E. dispar* strains

isolated from clinical samples [34]. However, only a few attempts have been made to apply the technique in clinical parasitology, particularly regarding helminth identification [25]. This tool presents an interesting alternative for laboratory identification of *Loa loa* microfilaria, because of its rapid execution and pathogen identification accuracy, especially in cases of co-infection between filarial. This study reports on the possibility of using the MALDI-TOF-MS technique to identify purified microfilariae of *L. loa*.

2. Methods

2.1. Study Site and Population

Samples were collected from March to April 2020 in three loiasis endemic villages in southeastern Gabon: Venez Voir, Mbouma-Ondama and Mvengue. These villages are located in the province of Haut-Ogooué, where the prevalence of *L. loa* microfilaremia infection is 18.1% [37]. The individuals who agreed to participate were assigned a code number and were interviewed during the interview. We collected demographic data (age, sex) and medical history (ocular passage of the worm [eye worm], calabar swelling, pruritus, etc.). Each participant signed an individual informed consent form. The study was approved by the National Ethics Committee of the country (PROT N00001/20/6/3/SG/CNE).

2.2. Blood Collection and Parasitological Analyses

Intravenous blood was collected in 4 ml EDTA (VWR International, France) tubes between 9 am and 2 pm. The collected blood was used for direct conventional parasitological diagnosis via the visualization of microfilariae in 10 µl of fresh blood under an optical microscope (×100 magnification). Parasites were identified by key morphological features, including the presence of a sheath, size (250 - 300 µm in length and 6 - 7 µm in width) and mobility (fast, snake-like movement) [38] [39].

2.3. Microfilarial Isolation and Purification

The purification and isolation of microfilaria were performed by using the protocol of Van Hoegaerden 1986 [40]. Briefly, the Iso-Osmotic Percoll Stock (SIP) was prepared by adding 9 volumes of Percoll and 1 volume of RPMI 1640. Gradient solutions containing 40%, 50% and 65% SIP were prepared and diluted with RPMI 1640 at 1× in 15 ml polystyrene tubes. Then, 2 ml of 65% gradient, 2 ml of the 50% gradient, and 2 ml of the 40% gradient were added. Over the gradient layers. A volume of 2 ml of whole blood from infected people was added. The 15 ml polystyrene tubes were centrifuged at 1000 × g for 20 minutes. After centrifugation, seven different layers appeared. *Loa Loa* microfilaria were located in the 3rd and 4th layers. These two microfilarial layers were introduced into a device consisting of a 5 µm pore filter and a syringe. Pressure was applied to the syringe so that the solution passed through the filter. The device was then disassembled, and the filters were incubated for 5 minutes in Petri dishes containing RPMI 1× to allow the

removal of the microfilariae. The microfilariae were then concentrated by centrifugation at 1000 × g for 3 minutes.

2.4. Protein Extraction Procedure

The microfilariae obtained from our samples were separated into 1, 10, 30, 40, 50, 100, 200, 300, 400, and 500 microfilariae per sample to test which concentration of parasite yielded the best protein spectra. The manipulation was performed under an inverted microscope [41] [42], and subsamples were placed into 1.5 ml Eppendorf tubes. Four procedures were compared to assess which procedure was the most effective for MALDI-TOF MS analyses. In procedure 1, the microfilaria was washed in alcohol, and after 10 minutes of centrifugation at 13000 rpm, the supernatant was removed, and the tube was air-dried. After drying, 10 µl of 70% formic acid (Sigma, France) was added to the tube, which was subsequently homogenized. The tube was air-dried for 5 min. After the incubation, 10 µl of 100% acetonitrile (Fluka, Buchs, Switzerland) was added to the tube, after which the tube was air-dried for another 5 min. The whole content of the tube was homogenized by quick centrifugation at 13,000 rpm. In procedure 2, the washing step was omitted, but the rest of the steps were unchanged. In protocol 3, the washing of samples was omitted, and the quantity of formic acid and acetonitrile was doubled (20 µl each). Finally, in protocol 4, the washing step was removed and the quantity of formic acid and acetonitrile was reduced by half; 5 µl was used instead of 10 µl of formic acid and acetonitrile (Figure 1).

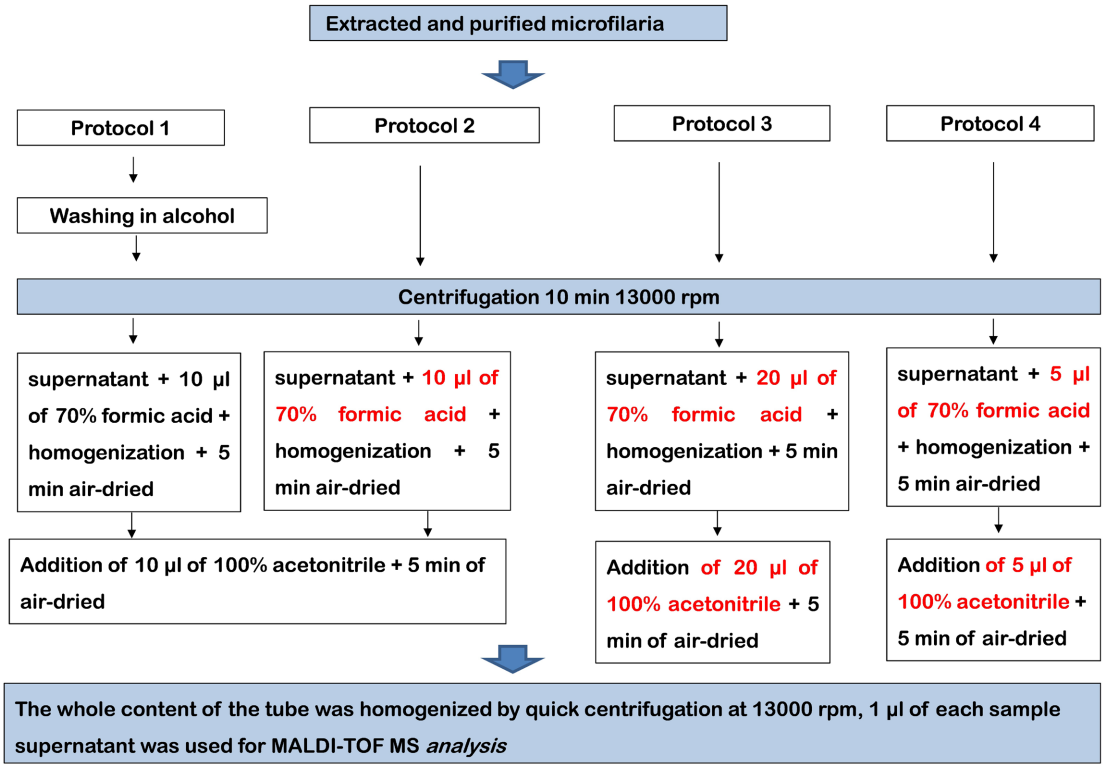

Figure 1. Flowchart of the extraction procedures for *Loa loa* microfilaria proteins.

2.5. MALDI-TOF MS Analysis

After protein extraction, 1 µl of each sample supernatant was deposited onto a MALDI-TOF MS target plate (Bruker Daltonics, Wissembourg, France). Ten replicates of each sample were spotted on the plate and air-dried. A negative extraction control was spotted on each run to detect contamination. After drying, the samples were covered with 1 µl of matrix solution composed of saturated alpha-cyano-4-hydroxycinnamic acid (Sigma), 50% acetonitrile, 2.5% trifluoroacetic acid (Aldrich, Dorset, UK) and high-performance liquid chromatography (HPLC)-grade water and air-dried. After drying, the target plate was placed in a Microflex LT™ spectrometer (Bruker Daltonics, Wissembourg, France) for analysis.

2.6. Mass Spectra Acquisition

Protein mass profiles were obtained using a Microflex LT™ MALDI-TOF mass spectrometer (Bruker Daltonics) and Flex Control™ software (Bruker Daltonics). The spectra were acquired after 240 shots in linear mode in ion-positive mode with a 337-nm nitrogen laser. The data were automatically acquired by the AutoXecute™ package of the FlexControl™ v2.4 software and exported into MALDI Biotyper™ v2.1 software. Only peaks with a signal/noise ratio of ≥3 were considered. The profiles of the spectra obtained were viewed using FlexAnalysis™ v.3.3 software and MALDI-Biotyper™ v.3.0 software (Bruker Daltonics) for data processing (smoothing, basic subtraction and peak selection) and cluster analysis. The quality of the obtained spectral profiles depends on the number of ribosomal marker peaks detected, the median relative intensity of ribosomal marker peaks, the sum of the intensity of all detected peaks, a high measurement precision, and reproducibility of peaks. Those criteria act as good proxies of spectral quality. The quality of the obtained spectral profiles depends on the number of ribosomal marker peaks detected, the median relative intensity of ribosomal marker peaks, the sum of the intensity of all detected peaks, a high measurement precision, and reproducibility of peaks. Those criteria act as good proxies of spectral quality [43].

2.7. Reference Spectral Library Construction

The spectra of each of the ten spots obtained for each *L. loa* sample were identified via comparison with the existing mass spectra library (MSL) via the "Start identification" function of the MaldiBiotyper™ software. For each reference sample, a main spectrum profile (MSP) was created using the automated function of MALDI-Biotyper™ software v.3.3. (Bruker Daltonics). The MSPs were included in a dedicated MSP database.

2.8. Mass Spectra Library (MSL)

To assess the technical reproducibility of the spectra obtained from the same biological sample, the log score values of each raw spectrum composing a main spectrum profile (MSP) were obtained by comparing each raw spectrum with its

proper MSP via the "Start identification" function of the MALDI Biotyper™ software. Moreover, to assess the biological reproducibility of the spectra obtained, the ten raw spectra obtained from each of the 8 *Loa loa* samples were queried against the MSL using the "Start identification" function of Maldi Biotyper™ software. The identification result showing the best log score value (LSV) among the 10 spots was recorded.

3. Results

3.1. Optical Microscopy Analysis

The *Loa loa* parasite was the only species found in the samples. After extraction and purification of *L. loa* worms. The microfilarial load in each blood sample varied from 5000 microfilariae to 25,000 microfilariae per microliter of blood (Table 1).

Table 1. Origin and *Loa loa* microfilarial load.

Sample	Origin	microfilarial load (mf/ml)
Loa 1	Venez-voir village	5000
Loa 2	Mbouma-Ondama village	14,250
Loa 3	Mvengue village	6460
Loa 4	Mvengue village	15,000
Loa 5	Mvengue village	25,000
Loa 6	Mvengue village	11,510
Loa 7	Mvengue village	12,500
Loa 8	Venez-voir village	10,000

3.2. Extraction Procedure Validation

Avoid Procedure 4 was used for protein extraction. The protocol efficiency was assessed by reading the extracted proteins on plates. The procedure validated was the one that produced interference-free spectra. The accuracy of the procedure was determined by reading 10 sample replicates on the spectrometer.

3.3. Mass Spectra Quality

The samples were divided into 1, 10, 30, 40, 50, 100, 200, 300, 400, and 500 microfilarial subsamples. Good resolution spectra and relatively high (>4000 a.u.) peak intensities were obtained from 400 microfilariae (Figure 2).

Comparison between the spectra obtained using protein extracts. Purified *Loa loa* microfilaria were counted and separated into 20, 30, 200, 400 microfilariae. The spectra were visualized using FlexAnalysis software (Figure 2).

3.4. MSP Database Validation

Technical reproducibility was assessed by analysing the LSVs obtained by comparing each of the ten spectra that were used to construct an MSP with this MSP.

We found that the technical reproducibility of the LSV ranged from 1.86 to 2.9, with a mean of 2.398 ± 0.046. Biological reproducibility was assessed by comparing distinct spectra acquired from a sample with the highest LSV acquired from a different sample best matched with their proper MSP for the reference isolates, with LSV ranging from 1.17 to 2.55 and a mean of 2.13 ± 0.03.

Figure 2. Mass spectra of the isolates of *Loa loa* microfilaria.

3.5. Blind Test and Cluster Analysis

In this study, we obtained 8 samples with good quality spectra from 80 spectra, which were subsequently used to create a database. The 80 spectra were subjected to a blind test, yielding an average log score value (LSV) of 2.13 according to the use of MALDI-Biotyper software v.3.3. To visualize MALDI-TOF MS profile similarities and distances, hierarchical clustering of the mass spectra of all tested species was performed using the dendrogram function of MALDI-Biotyper software v.3.3. Dendrogram analysis revealed specific clustering on distinct branches of *L. loa* according to species. *Loa loa* belonging to the same genus were grouped in the MSP dendrogram, but Loa 3 and Loa 8 were on a different branch (**Figure 3**).

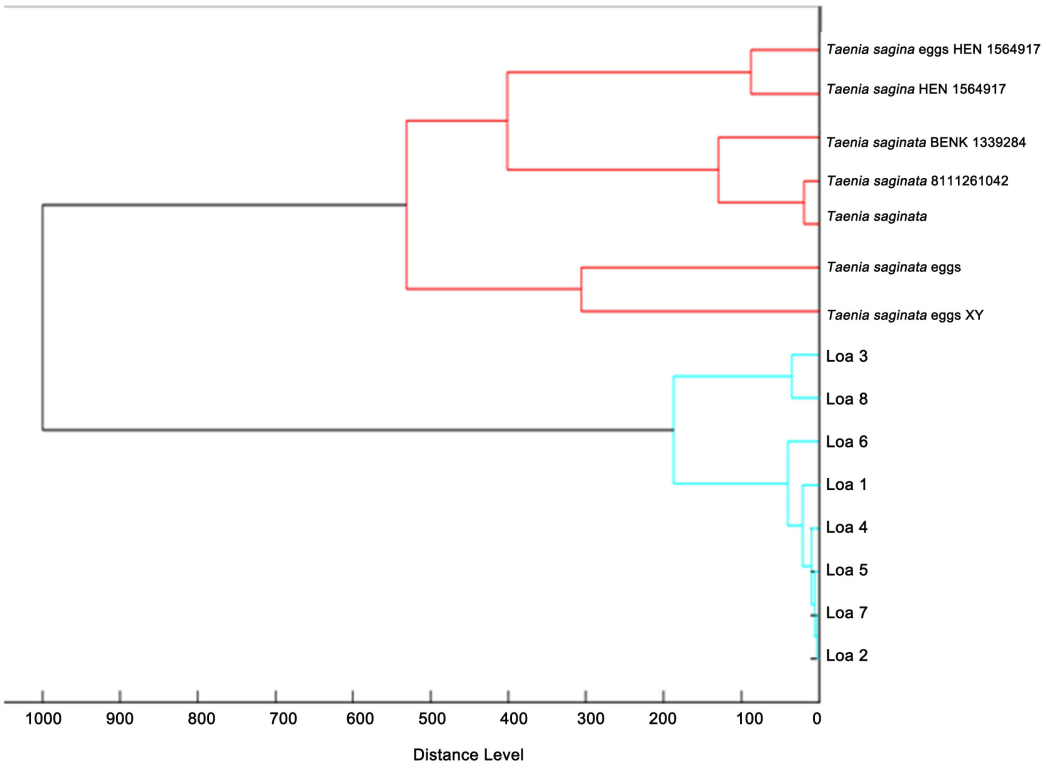

Figure 3. Dendrogram constructed using MALDI-Biotyper software v.3.3 illustrating the clear discrimination between the spectra acquired from 8 different samples of *L. loa* microfilaria and those acquired from proglottids and eggs of *Taenia saginata*.

4. Discussion

The diagnostic of *Loa loa* infection relies on conventional identification of parasites by microscopy. However, microscopy has a number of limitations; for example, the sensitivity of microscopy is affected by the low intensity of the infection. Over the past decade, matrix-assisted laser desorption-time-of-flight mass spectrometry (MALDI-TOF MS) has demonstrated its efficiency and accuracy in the identification of various microorganisms in clinical laboratories [18] [23] [28] [30] [35] [44]-[46]. Here, for the first time, we have used MALDI-TOF mass spectrometry for the identification of microfilariae isolated from *L. loa*. This is a pilot study; preliminary results are encouraging for the use of MALDI-TOF for the identification of microfilaria *Loa loa* microfilaria. Protein spectra were generated on the basis of repeatability and reproducibility in order to create species-specific MSPs for an internal database. The LSVs obtained had good technical reproducibility ranging from 1.86 to 2.9, with an average of 2.398 ± 0.046. Good biological reproducibility was also recorded, with a mean of 2.13 [1.17 - 2.55].

The experiments started with the validation of the protocol for extracting proteins from *L. loa* microfilaria. To achieve this goal, the quantities of formic acid and acetonitrile used to disrupt the cells were reduced compared to the standard protocol for extracting proteins from bacteria, which enabled us to have sufficiently concentrated proteins to observe signals after MALDI–TOF–MS analysis

[19] [44] [47]. The drying process enabled the crystallization of the sample. At this step, the mixture (sample + matrix) is subjected to a laser beam. The ions are separated during the migration according to their mass-to-charge ratio (time-of-flight). Once they reach a detector, a specific spectra profile is generated. Adequate spectral profiles were obtained from 400 *Loa loa* microfilaria (**Figure 2**). The unstable MS profiles obtained from 1 to around 400 microfilariae may be due to the body of the microfilaria, which comprises a fairly rigid cuticle that may be difficult to destroy. This was observed by Nebbak *et al.*, where the sturdiness of the cuticle for homogenization and the quick degradation of the filariae were factors that could decrease the abundance of proteins and peptides for the resulting MS spectra [48]. Also, Wendel *et al.* have assessed the potential effects of different storage conditions on the subsequent MALDI-TOF MS-based identification of helminths [49]. They concluded that samples can be reliably identified after prolonged storage in LC-MS-grade water, sodium chloride solution, and ethanol. For the identification of *Fasciola spp.* [50] and lice [51] 70% (v/v) ethanol was used as a storage solution, while studies on *Anisakis spp.* [52], *Dirofilaria spp.* and *Ascaris spp.* [53] employed a sodium chloride solution, which was sometimes even supplemented with antibiotics to prevent bacterial contamination [49]. Samples from our study were stored in distilled water, which probably damaged the worms present. We suggest that the higher the number of microfilariae, the more likely it was to find worms that were not damaged to extract good quality proteins to have high quality spectra. Nevertheless, the generated spectra were compared to an available reference spectra database available in our in-house database. We could identify all of our microfilaria samples by using our in-house mass spectra library. In our study, the database a spectrum that we had for Helminths were proglottids and eggs of *Taenia saginata*. In fact, in a previous study, spectral database for the identification of *Taenia saginata* proglottids was created [49]. Log score values range from zero to three and allow a good evaluation of the reproducibility between a queried spectrum and a reference spectrum [54]. This enables a good assessment of the reproducibility between an interrogated spectrum and a reference spectrum [47]. We obtained adequate (mean = 2.39, range [1.7 - 2.55]) log score values for matching between reference isolates and the proper MSP. The blind test resulted in the correct identification of *L. loa* microfilaria. Good biological reproducibility was recorded for 2.13% of the patients (mean 2,130 ± 0.03). Moreover, the technical reproducibility was good, with a score of 2.39 [1.86 to 2.9].

A dendrogram was constructed and showed a clear difference between the helminth species whose reference spectrum was included in our library of helminth mass spectra (**Figure 3**). The species *Loa loa* and *Taenia saginata* are well distinguished in the dendrogram. However, *Loa loa* microfilariae were classified into two distinct groups, with the main spectra profiles (MPS) of the microfilariae sampled in individuals 3 (Loa 3) and 8 (Loa 8) separated from the MPS of the other microfilariae. Individuals 3 and 8 are from the villages of Mvengue and Venezvoir. This finding led to the hypothesis that these patients were infected with a clone distinct from *Loa loa* microfilaria.

5. Limitations

This study showed that MALDI-TOF MS is a promising tool for the identification of *Loa loa*. In areas endemic to filarial species, it will help differentiate between worms that are morphologically relatively similar. Once the Loa-specific database is installed, new samples can be identified very quickly using a simple procedure. However, our study had several limitations, including the small sample size that likely affected the results. In addition, microfilariae of *L. loa* were counted manually by the investigator using reverse microscopy, which is tedious and takes time. In addition, the storage of the sample before analysis was not carefully monitored, which certainly resulted in the presence of damaged biological material, especially in small ones (1 to 100 microfilariae). The study was also limited by t independent samples independent set of samples to confirm its accuracy, reproducibility, and generalizability.

6. Conclusion

Our results position the MALDI-TOF MS technique as an innovative tool for identifying *Loa loa* microfilaria. MALDI-TOF MS technology has many advantages, including low consumption costs, easy sample preparation procedures, fast turnaround times and identification accuracy. Preliminary results are encouraging despite the limitations observed in the study. Interestingly, we observed phenotypic heterogeneity in the spectral profiles of *Loa loa* microfilarial proteins collected from individuals living in distinct regions. In perspective, the cohort needs to be broader, and further research is needed to identify the causes of this heterogeneity.

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Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

References

[1] Zouré, H.G.M., Wanji, S., Noma, M., Amazigo, U.V., Diggle, P.J., Tekle, A.H., *et al.* (2011) The Geographic Distribution of *Loa loa* in Africa: Results of Large-Scale Implementation of the Rapid Assessment Procedure for Loiasis (RAPLOA). *PLOS Neglected Tropical Diseases*, **5**, e1210. https://doi.org/10.1371/journal.pntd.0001210

[2] Whittaker, C., Walker, M., Pion, S.D.S., Chesnais, C.B., Boussinesq, M. and Basáñez, M. (2018) The Population Biology and Transmission Dynamics of *Loa loa*. *Trends in Parasitology*, **34**, 335-350. https://doi.org/10.1016/j.pt.2017.12.003

[3] Kelly-Hope, L., Paulo, R., Thomas, B., Brito, M., Unnasch, T.R. and Molyneux, D.

(2017) *Loa loa* Vectors *Chrysops* spp.: Perspectives on Research, Distribution, Bionomics, and Implications for Elimination of Lymphatic Filariasis and Onchocerciasis. *Parasites & Vectors*, **10**, Article No. 172. https://doi.org/10.1186/s13071-017-2103-y

[4] Akue, J., Eyang-Assengone, E. and Dieki, R. (2018) *Loa loa* Infection Detection Using Biomarkers: Current Perspectives. *Research and Reports in Tropical Medicine*, **9**, 43-48. https://doi.org/10.2147/rrtm.s132380

[5] Amir, M.M., Shaikh, A.S. and Ashraf, A. (2014) Subconjunctival *Loa loa* Worm: A Case Report. *Pakistan Journal of Ophthalmology*, **30**.

[6] Buell, K.G., Whittaker, C., Chesnais, C.B., Jewell, P.D., Pion, S.D.S., Walker, M., *et al.* (2019) Atypical Clinical Manifestations of Loiasis and Their Relevance for Endemic Populations. *Open Forum Infectious Diseases*, **6**, ofz417. https://doi.org/10.1093/ofid/ofz417

[7] Budge, P.J., Herbert, C., Andersen, B.J. and Weil, G.J. (2018) Adverse Events Following Single Dose Treatment of Lymphatic Filariasis: Observations from a Review of the Literature. *PLOS Neglected Tropical Diseases*, **12**, e0006454. https://doi.org/10.1371/journal.pntd.0006454

[8] Hoerauf, A., Pfarr, K., Mand, S., Debrah, A.Y. and Specht, S. (2011) Filariasis in Africa—Treatment Challenges and Prospects. *Clinical Microbiology and Infection*, **17**, 977-985. https://doi.org/10.1111/j.1469-0691.2011.03586.x

[9] Klion, A.D., Ottesen, E.A. and Nutman, T.B. (1994) Effectiveness of Diethylcarbamazine in Treating Loiasis Acquired by Expatriate Visitors to Endemic Regions: Long-Term Follow-Up. *Journal of Infectious Diseases*, **169**, 604-610. https://doi.org/10.1093/infdis/169.3.604

[10] Arrey-Agbor, D.B., Nana-Djeunga, H.C., Mogoung-Wafo, A.E., Mafo, M., Danwe, C. and Kamgno, J. (2018) Case Report: Probable Case of Spontaneous Encephalopathy Due to Loiasis and Dramatic Reduction of *Loa loa* Microfilariaemia with Prolonged Repeated Courses of Albendazole. *The American Journal of Tropical Medicine and Hygiene*, **99**, 112-115. https://doi.org/10.4269/ajtmh.17-0664

[11] Goussard, B., Ivanoff, B., Frost, E., Garin, Y. and Bourderiou, C. (1984) Age of Appearance of IgG, IgM, and IgE Antibodies Specific for *Loa loa* in Gabonese Children. *Microbiology and Immunology*, **28**, 787-792. https://doi.org/10.1111/j.1348-0421.1984.tb00734.x

[12] Fink, D.L., Kamgno, J. and Nutman, T.B. (2011) Rapid Molecular Assays for Specific Detection and Quantitation of *Loa loa* Microfilaremia. *PLOS Neglected Tropical Diseases*, **5**, e1299. https://doi.org/10.1371/journal.pntd.0001299

[13] Toure, F.S., Egwang, T.G., Wahl, G., Millet, P., Bain, O. and Georges, A.J. (1997) Species-specific Sequence in the Repeat 3 Region of the Gene Encoding a Putative *Loa loa* Allergen: A Diagnostic Tool for Occult Loiasis. *The American Journal of Tropical Medicine and Hygiene*, **56**, 57-60. https://doi.org/10.4269/ajtmh.1997.56.57

[14] TourÃ, F.S., Ungeheuer, M.N., Egwang, T.G. and Deloron, P. (1999) Use of Polymerase Chain Reaction for Accurate Follow-Up of *Loa loa* Experimental Infection in Mandrillus Sphinx. *The American journal of tropical medicine and hygiene*, **61**, 956-959. https://doi.org/10.4269/ajtmh.1999.61.956

[15] Drame, P.M., Meng, Z., Bennuru, S., Herrick, J.A., Veenstra, T.D. and Nutman, T.B. (2016) Identification and Validation of *Loa loa* Microfilaria-Specific Biomarkers: A Rational Design Approach Using Proteomics and Novel Immunoassays. *mBio*, **7**, e02132-15. https://doi.org/10.1128/mbio.02132-15

[16] Belkum, A.V., Welker, M., Pincus, D., Charrier, J. and Girard, V. (2017) Matrix-As-

sisted Laser Desorption Ionization Time-Of-Flight Mass Spectrometry in Clinical Microbiology: What Are the Current Issues? *Annals of Laboratory Medicine*, **37**, 475-483. https://doi.org/10.3343/alm.2017.37.6.475

[17] Clark, A.E., Kaleta, E.J., Arora, A. and Wolk, D.M. (2013) Matrix-Assisted Laser Desorption Ionization-Time of Flight Mass Spectrometry: A Fundamental Shift in the Routine Practice of Clinical Microbiology. *Clinical Microbiology Reviews*, **26**, 547-603. https://doi.org/10.1128/cmr.00072-12

[18] Fenselau, C. and Demirev, P.A. (2001) Characterization of Intact Microorganisms by MALDI Mass Spectrometry. *Mass Spectrometry Reviews*, **20**, 157-171. https://doi.org/10.1002/mas.10004

[19] Chen, X., Hou, X., Xiao, M., Zhang, L., Cheng, J., Zhou, M., *et al.* (2021) Matrix-assisted Laser Desorption/Ionization Time of Flight Mass Spectrometry (MALDI-TOF MS) Analysis for the Identification of Pathogenic Microorganisms: A Review. *Microorganisms*, **9**, Article 1536. https://doi.org/10.3390/microorganisms9071536

[20] Croxatto, A., Prod'hom, G. and Greub, G. (2012) Applications of MALDI-TOF Mass Spectrometry in Clinical Diagnostic Microbiology. *FEMS Microbiology Reviews*, **36**, 380-407. https://doi.org/10.1111/j.1574-6976.2011.00298.x

[21] Sy, I., Conrad, L. and Becker, S.L. (2022) Recent Advances and Potential Future Applications of MALDI-TOF Mass Spectrometry for Identification of Helminths. *Diagnostics*, **12**, Article 3035. https://doi.org/10.3390/diagnostics12123035

[22] Reuschenbach, M., Hohrenk-Danzouma, L.L., Schmidt, T.C. and Renner, G. (2022) Development of a Scoring Parameter to Characterize Data Quality of Centroids in High-Resolution Mass Spectra. *Analytical and Bioanalytical Chemistry*, **414**, 6635-6645. https://doi.org/10.1007/s00216-022-04224-y

[23] Strejcek, M., Smrhova, T., Junkova, P. and Uhlik, O. (2018) Whole-Cell MALDI-TOF MS versus 16S rRNA Gene Analysis for Identification and Dereplication of Recurrent Bacterial Isolates. *Frontiers in Microbiology*, **9**, Article 1294. https://doi.org/10.3389/fmicb.2018.01294

[24] Li, A., Higgs, J.M. and Austin, D.E. (2017) Chaotic Motion of Single Ions in a Toroidal Ion Trap Mass Analyzer. *International Journal of Mass Spectrometry*, **421**, 95-103. https://doi.org/10.1016/j.ijms.2017.06.005

[25] Feucherolles, M., Poppert, S., Utzinger, J. and Becker, S.L. (2019) MALDI-TOF Mass Spectrometry as a Diagnostic Tool in Human and Veterinary Helminthology: A Systematic Review. *Parasites & Vectors*, **12**, Article No. 245. https://doi.org/10.1186/s13071-019-3493-9

[26] Jakovljev, A. and Bergh, K. (2015) Development of a Rapid and Simplified Protocol for Direct Bacterial Identification from Positive Blood Cultures by Using Matrix Assisted Laser Desorption Ionization Time-of-Flight Mass Spectrometry. *BMC Microbiology*, **15**, Article No. 258. https://doi.org/10.1186/s12866-015-0594-2

[27] Carbonnelle, E., Mesquita, C., Bille, E., Day, N., Dauphin, B., Beretti, J., *et al.* (2011) MALDI-TOF Mass Spectrometry Tools for Bacterial Identification in Clinical Microbiology Laboratory. *Clinical Biochemistry*, **44**, 104-109. https://doi.org/10.1016/j.clinbiochem.2010.06.017

[28] Bader, O. (2016) Fungal Species Identification by MALDI-ToF Mass Spectrometry. In: Lion, T., Ed., *Human Fungal Pathogen Identification*, Springer, 323-337. https://doi.org/10.1007/978-1-4939-6515-1_19

[29] Sjöholm, M.I.L., Dillner, J. and Carlson, J. (2008) Multiplex Detection of Human Herpesviruses from Archival Specimens by Using Matrix-Assisted Laser Desorption Ionization-Time of Flight Mass Spectrometry. *Journal of Clinical Microbiology*, **46**, 540-

545. https://doi.org/10.1128/jcm.01565-07

[30] Yssouf, A., Almeras, L., Raoult, D. and Parola, P. (2016) Emerging Tools for Identification of Arthropod Vectors. *Future Microbiology*, **11**, 549-566. https://doi.org/10.2217/fmb.16.5

[31] Rakotonirina, A., Pol, M., Kainiu, M., Barsac, E., Tutagata, J., Kilama, S., *et al.* (2020) MALDI-TOF MS: Optimization for Future Uses in Entomological Surveillance and Identification of Mosquitoes from New Caledonia. *Parasites & Vectors*, **13**, Article No. 359. https://doi.org/10.1186/s13071-020-04234-8

[32] Sánchez-Juanes, F., Calvo Sánchez, N., Belhassen García, M., Vieira Lista, C., Román, R.M., Álamo Sanz, R., *et al.* (2022) Applications of MALDI-TOF Mass Spectrometry to the Identification of Parasites and Arthropod Vectors of Human Diseases. *Microorganisms*, **10**, Article 2300. https://doi.org/10.3390/microorganisms10112300

[33] Calderaro, A., Piergianni, M., Montecchini, S., Buttrini, M., Piccolo, G., Rossi, S., *et al.* (2016) MALDI-TOF Mass Spectrometry as a Potential Tool for *Trichomonas vaginalis* Identification. *BMC Infectious Diseases*, **16**, Article No. 261. https://doi.org/10.1186/s12879-016-1594-z

[34] Kassa, F.A., Shio, M.T., Bellemare, M., Faye, B., Ndao, M. and Olivier, M. (2011) New Inflammation-Related Biomarkers during Malaria Infection. *PLOS ONE*, **6**, e26495. https://doi.org/10.1371/journal.pone.0026495

[35] Cassagne, C., Pratlong, F., Jeddi, F., Benikhlef, R., Aoun, K., Normand, A.-., *et al.* (2014) Identification of Leishmania at the Species Level with Matrix-Assisted Laser Desorption Ionization Time-of-Flight Mass Spectrometry. *Clinical Microbiology and Infection*, **20**, 551-557. https://doi.org/10.1111/1469-0691.12387

[36] Mayer-Scholl, A., Murugaiyan, J., Neumann, J., Bahn, P., Reckinger, S. and Nöckler, K. (2016) Rapid Identification of the Foodborne Pathogen *Trichinella* spp. by Matrix-Assisted Laser Desorption/Ionization Mass Spectrometry. *PLOS ONE*, **11**, e0152062. https://doi.org/10.1371/journal.pone.0152062

[37] Akue, J.P., Nkoghe, D., Padilla, C., Moussavou, G., Moukana, H., Mbou, R.A., *et al.* (2011) Epidemiology of Concomitant Infection Due to *Loa loa* and *Mansonella perstans* in Gabon. *PLOS Neglected Tropical Diseases*, **5**, e1329. https://doi.org/10.1371/journal.pntd.0001329

[38] Mathison, B.A., Couturier, M.R. and Pritt, B.S. (2019) Diagnostic Identification and Differentiation of Microfilariae. *Journal of Clinical Microbiology*, **57**, e00706-19. https://doi.org/10.1128/jcm.00706-19

[39] Treviño, B., Zarzuela, F., Oliveira-Souto, I., Maturana, C.R., Serre-Delcor, N., Aznar, M.L., *et al.* (2023) Unexpected *Loa loa* Finding in an Asymptomatic Patient from the Gambia: A Case Report. *Open Forum Infectious Diseases*, **10**, ofad338. https://doi.org/10.1093/ofid/ofad338

[40] Bradford, M.M. (1976) A Rapid and Sensitive Method for the Quantitation of Microgram Quantities of Protein Utilizing the Principle of Protein-Dye Binding. *Analytical Biochemistry*, **72**, 248-254. https://doi.org/10.1016/0003-2697(76)90527-3

[41] Zofou, D., Fombad, F.F., Gandjui, N.V.T., Njouendou, A.J., Kengne-Ouafo, A.J., Chounna Ndongmo, P.W., *et al.* (2018) Evaluation of *in Vitro* Culture Systems for the Maintenance of Microfilariae and Infective Larvae of *Loa loa*. *Parasites & Vectors*, **11**, Article No. 275. https://doi.org/10.1186/s13071-018-2852-2

[42] Rojas, A., Rojas, D., Montenegro, V.M. and Baneth, G. (2015) Detection of *Dirofilaria immitis* and Other Arthropod-Borne Filarioids by an HRM Real-Time QPCR, Blood-Concentrating Techniques and a Serological Assay in Dogs from Costa Rica. *Parasites & Vectors*, **8**, Article No. 170. https://doi.org/10.1186/s13071-015-0783-8

[43] Cuénod, A., Foucault, F., Pflüger, V. and Egli, A. (2021) Factors Associated with MALDI-TOF Mass Spectral Quality of Species Identification in Clinical Routine Diagnostics. *Frontiers in Cellular and Infection Microbiology*, **11**, Article 646648. https://doi.org/10.3389/fcimb.2021.646648

[44] Matsuda, N., Matsuda, M., Notake, S., Yokokawa, H., Kawamura, Y., Hiramatsu, K., *et al.* (2012) Evaluation of a Simple Protein Extraction Method for Species Identification of Clinically Relevant Staphylococci by Matrix-Assisted Laser Desorption Ionization-Time of Flight Mass Spectrometry. *Journal of Clinical Microbiology*, **50**, 3862-3866. https://doi.org/10.1128/jcm.01512-12

[45] Yssouf, A., Socolovschi, C., Leulmi, H., Kernif, T., Bitam, I., Audoly, G., *et al.* (2014) Identification of Flea Species Using MALDI-TOF/MS. *Comparative Immunology, Microbiology and Infectious Diseases*, **37**, 153-157. https://doi.org/10.1016/j.cimid.2014.05.002

[46] Li, Y., Shan, M., Zhu, Z., Mao, X., Yan, M., Chen, Y., *et al.* (2019) Application of MALDI-TOF MS to Rapid Identification of Anaerobic Bacteria. *BMC Infectious Diseases*, **19**, Article No. 941. https://doi.org/10.1186/s12879-019-4584-0

[47] Williams, T.L., Andrzejewski, D., Lay, J.O. and Musser, S.M. (2003) Experimental Factors Affecting the Quality and Reproducibility of MALDI TOF Mass Spectra Obtained from Whole Bacteria Cells. *Journal of the American Society for Mass Spectrometry*, **14**, 342-351. https://doi.org/10.1016/s1044-0305(03)00065-5

[48] Nebbak, A. and Almeras, L. (2020) Identification of *Aedes* Mosquitoes by MALDI-TOF MS Biotyping Using Protein Signatures from Larval and Pupal Exuviae. *Parasites & Vectors*, **13**, Article No. 161. https://doi.org/10.1186/s13071-020-04029-x

[49] Wendel, T.P., Feucherolles, M., Rehner, J., Poppert, S., Utzinger, J., Becker, S.L., *et al.* (2021) Evaluating Different Storage Media for Identification of *Taenia saginata* Proglottids Using MALDI-TOF Mass Spectrometry. *Microorganisms*, **9**, Article 2006. https://doi.org/10.3390/microorganisms9102006

[50] Sy, I., Margardt, L., Ngbede, E.O., Adah, M.I., Yusuf, S.T., Keiser, J., *et al.* (2020) Identification of Adult *Fasciola* spp. Using Matrix-Assisted Laser/Desorption Ionization Time-of-Flight (MALDI-TOF) Mass Spectrometry. *Microorganisms*, **9**, Article 82. https://doi.org/10.3390/microorganisms9010082

[51] Ouarti, B., Laroche, M., Righi, S., Meguini, M.N., Benakhla, A., Raoult, D., *et al.* (2020) Development of MALDI-TOF Mass Spectrometry for the Identification of Lice Isolated from Farm Animals. *Parasite*, **27**, Article No. 28. https://doi.org/10.1051/parasite/2020026

[52] Marzano, V., Pane, S., Foglietta, G., Levi Mortera, S., Vernocchi, P., Onetti Muda, A., *et al.* (2020) Mass Spectrometry Based-Proteomic Analysis of *Anisakis* spp.: A Preliminary Study Towards a New Diagnostic Tool. *Genes*, **11**, Article 693. https://doi.org/10.3390/genes11060693

[53] Nagorny, S.A., Aleshukina, A.V., Aleshukina, I.S., Ermakova, L.A. and Pshenichnaya, N.Y. (2019) The Application of Proteomic Methods (MALDI-Toff MS) for Studying Protein Profiles of Some Nematodes (Dirofilaria and Ascaris) for Differentiating Species. *International Journal of Infectious Diseases*, **82**, 61-65. https://doi.org/10.1016/j.ijid.2019.02.047

[54] Fall, F.K., Laroche, M., Bossin, H., Musso, D. and Parola, P. (2021) Performance of MALDI-TOF Mass Spectrometry to Determine the Sex of Mosquitoes and Identify Specific Colonies from French Polynesia. *The American Journal of Tropical Medicine and Hygiene*, **104**, 1907-1916. https://doi.org/10.4269/ajtmh.20-0031

Abbreviations

MS: Mass spectrometry

MALDI-TOF MS: Matrix-assisted Laser Desorption Ionization-Time of Flight mass spectrometry

MSL: Mass Spectra Library

MSP: Main Spectra Profile

SIP: Iso-Osmotic Percoll Stock

LSV: Log score value