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Institut de Recherche pour le Développement – UMI 233 – INSERM U1175 – Université de Montpellier

# Développement de stratégies alternatives pour l'élimination des filarioses en Afrique centrale

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**Le 08 novembre 2021**

Sous la direction **de Sébastien BERTOUT**
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## LISTE DES PUBLICATIONS ET COMMUNICATIONS

### Articles publiés :

1. **Campillo JT**, Awaca-Uvon NP, Tambwe JP, Kuyangisa-Simuna G, Vlaminck J, Weil GJ, Boussinesq M, Chesnais CB and Pion SDS. A strong effect of individual compliance with mass drug administration for lymphatic filariasis on sustained clearance of soil-transmitted helminth infections. *Parasites Vectors* 14, 310 (2021)

2. **Campillo JT**, Boussinesq M, Bertout S, Faillie JL, Chesnais CB. Serious adverse reactions associated with ivermectin: A systematic pharmacovigilance study in sub-Saharan Africa and in the rest of the World. *PLoS Negl Trop Dis*. 2021 Apr 20;15(4)

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7. *Hors doctorat* : Chesnais CB, Bizet C, **Campillo JT**, Njamnshi WY, Bopda J, Nwane P, Pion SD, Njamnshi AK, Boussinesq M. A second population-based cohort study in Cameroon confirms the temporal relationship between onchocerciasis and epilepsy. *Open Forum Infect Dis.* 2020 Jun 2;7(6)

8. *Hors doctorat* **: Campillo JT**, Chabot EB, Awaca-Uvon NP, Tambwe JP, Kuyangisa-Simuna G, Boussinesq M, Chesnais CB, Pion SD. Effect of lymphatic filariasis and hookworm infection on pregnancy course and outcome in women living in the Democratic Republic of the Congo. *Am J Trop Med Hyg*. 2021 May 3;104(6):2074–81.

## Communications :

1. **Orale** Increased benefit of semi-annual treatment with albendazole alone to clear individual infection with *Wuchereria bancrofti*, when compared with annual treatment: longitudinal analysis from two cohort data from Central Africa, *ASTMH 69th Annual Meeting* (November 15-19, 2020) - *Virtual*

2. **Poster** Effect of lymphatic filariasis and hookworm infection on pregnancy course and outcome in women living in the Democratic Republic of the Congo, *ASTMH 68th Annual Meeting* (November 20-24, 2019) – *National Harbor, USA*

3. **Poster** A longitudinal analysis of data from two cohorts shows that mass drug administration of albendazole for filariasis elimination in Central Africa has a dose-related beneficial effect on soil-transmitted helminths, *ASTMH 69th Annual Meeting* (November 15-19, 2020) - *Virtual*

4. **Poster** Effets indésirables graves associés à l'ivermectine : une étude systématique de pharmacovigilance en Afrique Sub-saharienne et dans le reste du monde, JNI 22ème édition (30 août–2 septembre 2021) - *Montpellier*

5. **Poster** Évaluation de l'efficacité et de la tolérance du lévamisole chez des patients microfilarémiques à *Loa loa* : essai clinique contrôlé randomisé à dose et intensité d'infection croissantes, JNI 22ème édition (30 août–2 septembre 2021) - *Montpellier*

6. **Poster** Les effets indésirables associés au lévamisole varient en fonction de ses indications et de son mésusage : une étude systématique de pharmacovigilance, JNI 22ème édition (30 août–2 septembre 2021) – *Montpellier*

7. **Poster** Safety and efficacy of levamisole in loiasis: a randomized, placebo-controlled, double-blind clinical trial with ascending-dose and ascending-parasite density, SFP (28-29 octobre 2021) - Lyon

8. **Écrite** Les encéphalopathies graves dues à l'ivermectine sont-elles circonscrites aux zones d'endémie à l'onchocercose et la loase ? *Bulletin d'Informations de Pharmacologie Clinique de la région Occitanie,* 2020 ;27(3):37-58

9. **Écrite** Lévamisole : des effets graves immunomédiées peuvent survenir ! *Bulletin d'Informations de Pharmacologie Clinique de la région Occitanie*, 2020 ;27(4):70-98

# TABLE DES MATIERES

# Table des illustrations - Figures

# TABLE DES ILLUSTRATIONS - TABLES

## LISTE DES ABREVIATIONS

| | |
|---|---|
| ADN | Acide Désoxyribonucléique |
| ADR | Adverse Drug Reaction |
| AGEP | Acute Generalized Exanthematous Pustulosis |
| AIC | Akaike Information Criterion |
| ALB | Albendazole |
| AMM | Autorisation de Mise sur le Marché |
| ANSM | Agence Nationale de Sécurité du Médicament |
| APOC | African Programme for Onchocerciasis Control |
| aROR | Adjusted Reporting Odds Ratios |
| ATC | Anatomical Therapeutic Chemical |
| CBS | Calibrated Blood Smears |
| CDTI | Community-Directed Treatment with Ivermectin |
| CFA | Circulating Filarial Antigenemia |
| CI | Confidence Intervals |
| CMFL | Community Microfilarial Load |
| COFRAC | Comité Français d'Accréditation |
| COMT | Catechol-O-Methyl Transferase |
| COVID-19 | Coronavirus Disease 19 |
| DA | Diéthylcarbamazine et Albendazole |
| DALYs | Disability-Adjusted Life Years |
| DEC | Diéthylcarbamazine |
| DMF | Densité microfilarienne |
| DOLF | Death to Onchocerciasis and Lymphatic Filariasis |
| DRESS | Drug Reaction with Eosinophilia and Systemic Symptoms |
| ELISA | Enzyme-Linked Immunosorbent Assay |
| EPG | Eggs Per Gram |
| ESG | Effets Secondaires Graves |
| ESPEN | Expanded Special Project for Elimination of Neglected Tropical Diseases |
| FDA | Food and Drug Administration |
| FTS | Filarial Test Strip |
| HD | Health District |
| IC | Intervalles de confiance |
| ICC | Intraclass Correlation Coefficient |
| ICH | International Conference on Harmonization |
| ICSR | Individual Case Safety Report |
| ICT | Test Immuno-Chromatographique sur Carte |
| IDA | Ivermectine, Diéthylcarbamazine et Albendazole |
| IELP | Technique d'Immunoélectrophorèse |
| IFI | Immunofluorescence indirecte |
| IR | Incidence Rates |
| IRD | Institut de Recherche pour le Développement |
| IRM | Imagerie par Résonnance Magnétique |
| IRR | Incidence Rate Ratios |
| IVM | Ivermectin |
| LAMP | Loop-Mediated Isothermal Amplification |

| | |
|---|---|
| LEV | Levamisole |
| LF | Lymphatic filariasis |
| LIPS | Luciferase Immunoprecipitation Systems |
| MA | Market authorization |
| MAO-A | Monoamine Oxidase type A |
| MDA | Mass Drug Administration |
| MedDRA | Medical Dictionary for Regulatory Activities |
| Mf | Microfilariae |
| MFD | Microfilarial Densities |
| MGG | May Grunwald Giemsa |
| MTN | Maladies Tropicales Négligées |
| NTD | Neglected Tropical Diseases |
| OAE | Onchocerciasis-Associated Epilepsy |
| OCP | Onchocerciasis Control Programme |
| OMS | Organisation Mondiale de la Santé |
| PCR | Polymerase Chain Reaction |
| PLERM | Possible/probable *Loa loa* Encephalopathy temporally Related to Mectizan |
| PT | Preferred Term |
| PWE | People With Epilepsy |
| PY | Person-Years |
| RAPLOA | Rapid Assessment Procedure for Loiasis |
| RCP | Résumé des Caractéristiques du Produit |
| REMO | Rapid Epidemiological Mapping of Onchocerciasis |
| ROR | Reporting Odds Ratio |
| ROW | Rest Of the World |
| sADRs | Serious Adverse Drug Reactions |
| SAE | Serious Adverse Events |
| SARS | Severe Acute Respiratory Syndrome |
| SCE | Suspected Cases of Epilepsy |
| SFBC | Société Française de Biochimie Clinique |
| SJS | Syndrome de Stevens Johnson |
| SOC | System Organ Class |
| SSA | Sub-Saharan Africa |
| STH | Soil-Transmitted Helminth |
| TEN | Toxic Epidermal Necrolysis |
| TIDC | Traitement par Ivermectine sous Directives Communautaires |
| TNnT | Test & not Treat |
| TNT | Test & Treat |
| VON | Valeur Prédictive Négative |
| WHO | World Health Organization |

# 1. Introduction

## 1.1 Maladies tropicales négligées

Le concept de maladies tropicales négligées (MTNs) a émergé suite à une réunion d'un groupe de travail de l'Organisation Mondiale de la Santé (OMS) à Berlin en 2003 (Organisation Mondiale de la Santé 2004). Il regroupe actuellement 20 maladies transmissibles partageant des caractéristiques communes comme leur grande morbidité et leur répartition géographiquement limitée aux pays pauvres d'Asie, d'Afrique et d'Amérique latine. On y trouve notamment des infections bactériennes (lèpre, trachome, pian et ulcère de Buruli), des infections virales (dengue et rage), les morsures de serpent et des infections parasitaires (trypanosomose, leishmaniose, cysticercose, échinococcose, dracunculose, filariose lymphatique, onchocercose, bilharziose et les nématodoses intestinales).

Le terme de « négligées » renvoie au fait que ces maladies ont pendant longtemps été ignorées par le pouvoir politique et les bailleurs internationaux et que très peu de fonds leur ont été consacrés par comparaison au VIH, à la tuberculose et au paludisme. Associées à la pauvreté et l'insalubrité, les MTNs touchent presque exclusivement des populations pauvres vivant dans des pays en développement au climat tropical ou subtropical. Il est estimé qu'environ 1 milliard de personnes dans le monde sont actuellement atteintes par au moins une de ces MTNs (Organisation Mondiale de la Santé 2010). Ces maladies, bien qu'ayant des manifestations cliniques différentes, ont toutes la capacité d'engendrer des incapacités sévères ou des lésions permanentes. Elles partagent toutes un grand nombre de personnes à risque d'exposition. On les considère comme des maladies chroniques, persistantes etqui, cependant, ont un impact considérable sur la productivité économique des pays touchés et sur la stigmatisation des malades.

Face à ces maladies, l'OMS a défini 5 stratégies d'action (Organisation Mondiale de la Santé 2014) :

- La chimioprophylaxie préventive à large échelle, qui se concentre sur les maladies pour lesquelles une stratégie de traitement efficace et sûre existe : la filariose lymphatique, l'onchocercose, la bilharziose et les nématodoses intestinales ;
- La prise en charge intensifiée des cas, qui se concentre sur les maladies pour lesquelles le traitement à large échelle n'est pas possible et pour lesquelles il n'existe pas d'outils de contrôle appropriés : l'ulcère de Buruli, la trypanosomose, la leishmaniose, les morsures de serpents et le pian ;
- La lutte antivectorielle, qui repose sur l'amélioration de l'efficacité, du rapport coût-efficacité, du respect écologique et de la durabilité de la lutte contre les vecteurs des MTNs à transmission vectorielle : la trypanosomose, la leishmaniose, la filariose lymphatique, l'onchocercose, la dengue et la dracunculose ;
- La garantie d'un accès à l'eau sans risque sanitaire et la promotion de l'hygiène ;;
- La mise en œuvre de mesures de santé publique vétérinaire à la fois pour les animaux domestiques et les animaux sauvages.

Ainsi, certaines de ces MTN font l'objet, depuis plusieurs décennies, de programmes de lutte ayant pour objectif d'éradiquer la maladie ou au moins d'en stopper la transmission.

Parmi les infections parasitaires comptées au rang de MTNs, nous trouvons des filarioses : l'onchocercose et la filariose lymphatique. Une autre filariose, la loase, ne fait actuellement pas partie des MTNs. Les travaux présentés dans cette thèse portent sur ces trois filarioses.

## 1.2 Filarioses

Les filarioses sont des parasitoses causées par des nématodes (vers ronds), elles sont transmises par des insectes vecteurs. Il existe de multiples filarioses dans le monde animal mais seulement trois d'entre elles vont nous intéresser tout au long de ce travail de thèse : la filariose lymphatique, l'onchocercose et la loase.

### 1.2.1 Filariose lymphatique

#### *1.2.1.1 Présentation*

Deux genres de parasites peuvent provoquer la filariose lymphatique : le genre *Wuchereria* (*Wuchereria bancrofti*), largement majoritaire et essentiellement présent dans les zones tropicales et subtropicales d'Afrique, d'Asie, du Pacifique et des Amériques et le genre *Brugia* (*Brugia malayi* et *Brugia timori*), moins répandu, présent uniquement en Asie du Sud et de l'Est.

La transmission de la filariose lymphatique est due aux moustiques appartenant aux genres *Anopheles*, *Aedes*, *Culex* ou *Mansonia* qui sont les hôtes intermédiaires du parasite tandis que l'Homme est l'hôte définitif. Pendant un repas sanguin, le moustique ingurgite les embryons du ver, appelés microfilaires, dans le sang de l'hôte définitif. Les microfilaires évoluent dans le moustique en larves L2 non infestantes puis en larves L3 infestantes pour l'Homme. Les larves L3 sont transmises à l'homme lors d'un second repas sanguin. Par la suite, une partie des larves L3 poursuivent leur évolution et migrent vers les vaisseaux lymphatiques où elles muent en larves L4. En quelques mois, elles vont se transformer en adultes qui vont s'accoupler et produire des microfilaires. Ces microfilaires vont circuler en permanence dans la lymphe et périodiquement dans le sang (figure 1, source : CDC).

Les vers adultes femelles mesurent 8 à 10 centimètres tandis que les mâles mesurent environ 4 centimètres et les microfilaires 300 micromètres. Les vers adultes vivent pendant environ 6 ans dans le système lymphatique de l'hôte définitif. Les vers adultes ont la particularité d'héberger des bactéries intracellulaires appelées *Wolbachia*.

Chaque espèce de moustiques est spécifique d'une ou plusieurs espèces de parasites et chaque espèce de parasite possède une périodicité particulière, c'est-à-dire une période préférentielle où les microfilaires seront présentes dans le sang périphérique de l'hôte définitif. Cela résulte directement d'un phénomène de coévolution qui a abouti à l'adaptation du comportement des vecteurs afin d'être en phase avec la périodicité des microfilaires dans le sang périphérique de l'hôte humain. Ainsi, si le parasite *Wuchereria bancrofti* est transmis par le genre *Anopheles*, *Culex* ou *Mansonia*, la périodicité sera nocturne tandis que s'il s'agit du genre *Aedes*, la périodicité sera sub-nocturne.

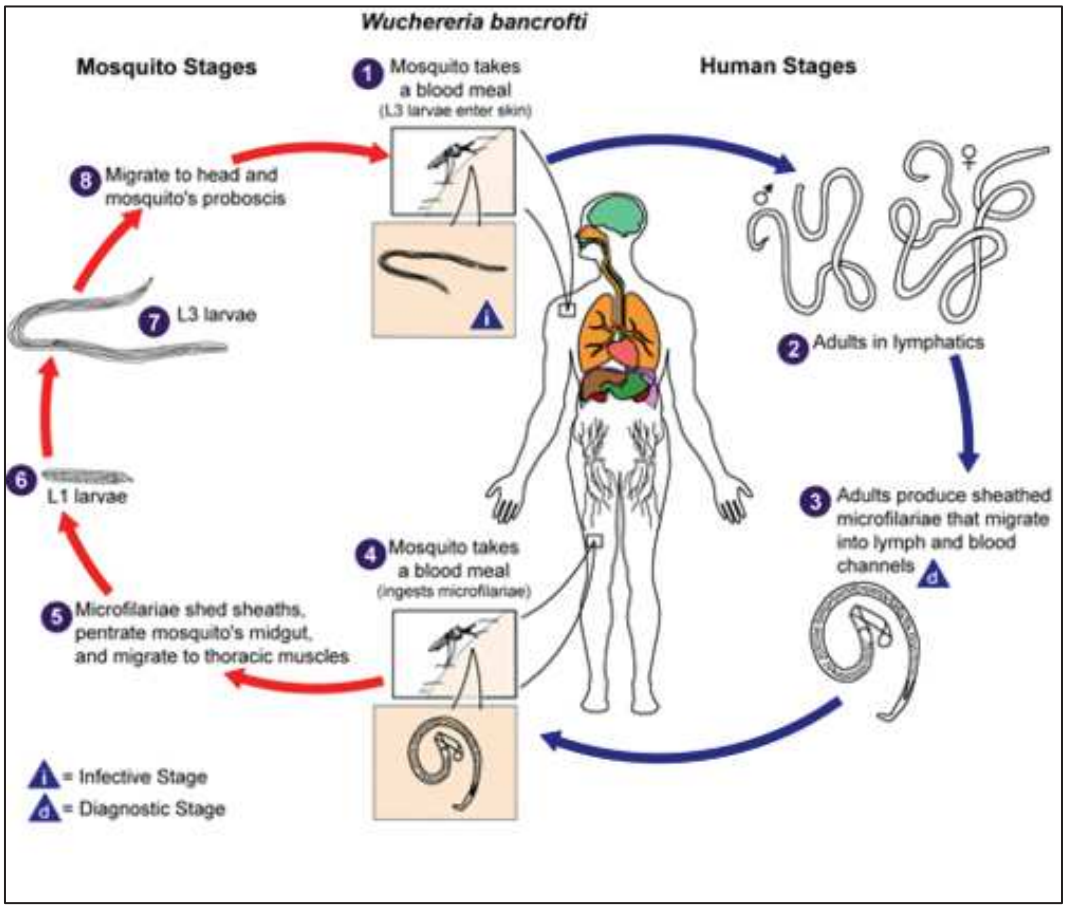



**Figure 1. Cycle parasitaire de la filariose lymphatique à Wuchereria bancrofti**

### *1.2.1.2 Épidémiologie*

Après le paludisme, la filariose lymphatique est la maladie parasitaire à transmission vectorielle la plus importante par les handicaps physiques et les conséquences socio-économiques qu'elles engendrent. L'OMS estime qu'environ 893 millions de personnes continuent d'être exposés à la filariose lymphatique en 2021 au sein de 49 pays différents, que 51 millions de personnes étaient infectées par la filariose lymphatique en 2018 dont 50 millions en Afrique subsaharienne et que 36 millions de personnes ont développé des manifestations chroniques (figure 2, *source : relevé épidémiologique hebdomadaire n°45 - 2020, OMS).*

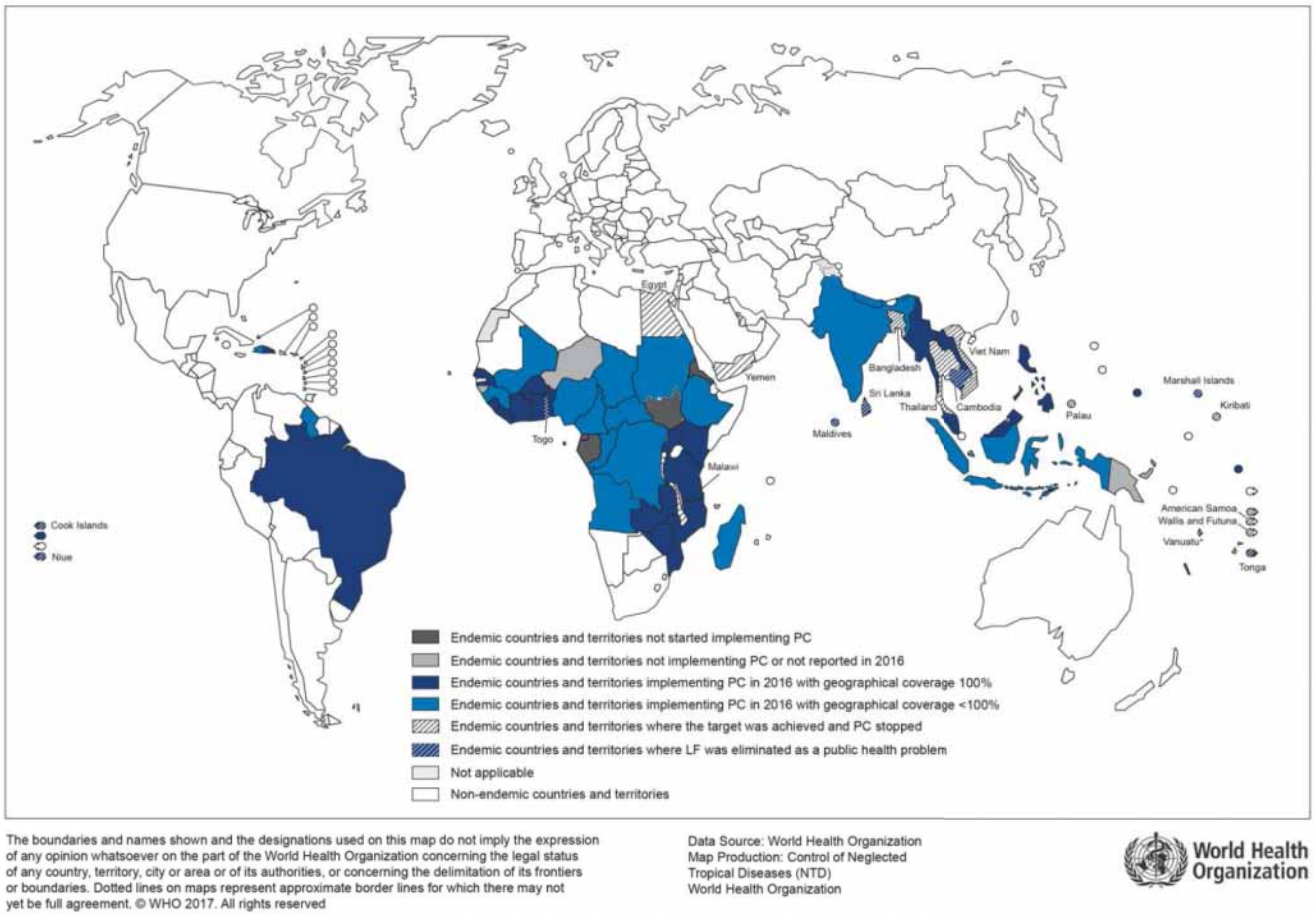



**Figure 2. Distribution mondiale de la filariose lymphatique et statuts des pays endémiques concernant la mise en place des mesures d'administration de masse de médicaments à visée préventive (PC) en 2016**

### *1.2.1.3 Manifestations cliniques*

On considère deux types de manifestations cliniques de la filariose lymphatique : les formes aigües et les formes chroniques que l'on peut distinguer par les mécanismes physiopathologiques qui les entrainent. Ces mécanismes impliquent les vers adultes et expliquent la majorité des symptômes de la filariose lymphatique :

(i) La mort des vers adultes entraine des inflammations du système lymphatique et des atteintes de la microcirculation lymphatique. Les symptômes qui s'en suivent sont aigus. On note notamment : les adénolymphangites des membres, les dermato-lymphangio-adénites des membres ou de l'appareil génital et enfin, dans de rares cas, une forme pulmonaire grave associant crises asthmatiformes, asthénie, fièvre et perte de poids, appelée poumon éosinophile causé par à une hypersensibilité aux microfilaires.

(ii) Les vers adultes vivants sont capables de provoquer des obstructions du système lymphatique engendrant des dilatations lymphatiques, des fuites de lymphe et des accumulations de celle-ci en dehors du système lymphatique. On observe des symptômes chroniques de type hydrocèles, orchi-épididymites, varices lymphatiques, chyluries et lymphœdèmes. Enfin, si ces obstructions persistent, les tissus sous-cutanés peuvent se fragiliser et devenir un terrain propice au développement de surinfections bactériennes et mycosiques. Au stade ultime, on parlera d'éléphantiasis.

#### *1.2.1.4 Méthodes diagnostiques*

### A. Diagnostic clinique et diagnostic direct

Le diagnostic direct clinique est délicat. Cependant, le recours à l'IRM et à l'échographie ganglionnaire ou lymphatique peut permettre de visualiser les atteintes lymphatiques, les vers adultes et les atteinte testiculaires avec un signe caractéristique de la filariose scrotale appelé « danse filarienne » (Chanteau et al. 1994; Amaral et al. 1994; Noroes et al. 1996; Carme and Esterre 2012). Le tableau d'éléphantiasis peut toutefois permettre d'orienter le diagnostic vers la filariose lymphatique. Il faudra cependant évoquer le diagnostic différentiel de podoconiose (obstruction lymphatique chronique touchant les sujets marchant pieds nus en zone volcanique) en cas d'éléphantiasis des membres inférieurs asymétrique et s'arrêtant au niveau des genoux (Gahlinger 2021).

La méthode diagnostique de référence est parasitologique et repose sur la réalisation de la goutte épaisse, qui correspond à l'étalement sur une lame porte-objet d'une quantité calibrée (de 20 à 100 µL) de sang capillaire prélevé au bout du doigt. Celle-ci permet l'identification microscopique de microfilaires et dont la morphologie est spécifique (longueur de 250 à 310 µm, présence d'une gaine et de deux noyaux : un sub-terminal et un terminal). Selon l'espèce de filariose lymphatique, le prélèvement doit être réalisé pendant des heures spécifiques à la périodicité des microfilaires : périodicité nocturne pour l'infection à *W. bancrofti* et périodicité diurne pour l'infection à *B. malayi*. Une fois la lame porte-objet déshémoglobinisée et colorée au Giemsa dans les 24 heures suivant l'étalement, un dénombrement des microfilaires est réalisé par un microscopiste expérimenté. Cette méthode permet d'obtenir la densité microfilarienne individuelle, généralement exprimée en nombre de microfilaires par millilitre de sang. Une limite importante de cette technique de microscopique est l'existence d'infection à filariose

lymphatique qu'on appelle occulte. C'est-à-dire que les individus infectés ne possèdent aucune microfilaire dans le prélèvement réalisé. Ceci représentant environ un tiers des individus infectés. Des techniques de concentration des microfilaires (leucoconcentration, technique de Knott et filtration membranaire) peuvent également être utilisées.

Ces méthodes directes présentent l'inconvénient de sous-estimer la prévalence de la microfilarémie si la densité des microfilaires est faible (limite de détection située entre 15 et 60 microfilaires par millilitre (Haute Autorité de Santé 2018)).

Des méthodes diagnostiques innovantes ont été mises au point comme la détection d'antigènes filariens circulants spécifiques des vers adultes. Ces tests reposent sur des méthodes ELISA réalisés en laboratoire à partir des antigènes AD12 et Og4C3 (Weil et al. 1987; Chanteau et al. 1994; More and Copeman 1990). Ils sont plutôt utilisés dans le cadre de la recherche. Devant les difficultés évidentes à réaliser ces tests pour les pays concernés, plus récemment, de nouvelles techniques ont été développés pour mettre en place des tests utilisables directement sur le terrain, avec un résultat en seulement 10 minutes. Le premier test rapide développé était le test immuno-chromatographique sur carte (ICT) (Binax Now Filariasis ICT test, Alere, Portland, ME) (Weil et al. 1997). En, 2013, le test sur bandelette appelée Filarial Test Strip (FTS) (Weil et al. 2013) reposant sur la même technologie que le test ICT, a été développé. Ce dernier, du fait d'une sensibilité supérieure (Chesnais et al. 2017), un prix plus faible et une logistique de transport plus adaptée (pas de nécessité de stockage au froid), a remplacé le test ICT dans le cadre de programmes de lutte contre la filariose lymphatique. Cette méthode antigénique repose sur l'utilisation d'anticorps monoclonaux spécifiques dirigés contre l'antigène AD12 de *W. bancrofti*. Elle a plusieurs avantages : elle est plus sensible que la goutte épaisse calibrée pour diagnostiquer une infection à *W. bancrofti*, elle permet le diagnostic de l'infection occulte, il n'y a pas besoin de respecter la périodicité des microfilaires, elle est plus simple et rapide d'utilisation, seulement une dizaine de minutes sont nécessaires pour poser un diagnostic. Cependant, des résultats récents ont montré l'existence d'une réaction croisée si l'individu testé présente une forte microfilarémie à *Loa loa* (Bakajika et al. 2014; Pion et al. 2016; Wanji et al. 2016). De plus, ces tests rapides ne permettent pas de diagnostiquer une infection à *B. malayi*.

## B. Diagnostic indirect

Une autre méthode immunologique de détection repose sur l'identification d'anticorps circulants (IgG4 anti-Wb123). De manière similaire aux tests antigéniques, ces tests peuvent être réalisés toute la journée, indépendamment de la périodicité des microfilaires. Il semblerait que ces tests soient extrêmement spécifiques à l'infection à *W. bancrofti*. Différents tests existent : des tests immuno-enzymatiques ELISA réservés aux laboratoires et des tests « cassettes » (Wb123+Ov16), encore en cours d'évaluation. Ces outils de détection d'anticorps permettent la surveillance de la transmission de l'infection et l'évaluation des campagnes d'administration massive de médicaments (Alhassan et al. 2015).

Des techniques de détection d'ADN par réaction de polymérisation en chaine (PCR) peuvent permettre la détection de *W. bancrofti* ou de *B. malayi* dans les échantillons de sang. La cible d'amplification utilisée pour *W. bancrofti* est la séquence LDR. Ces techniques de PCR sont sensibles et spécifiques mais sont, pour l'instant, réservées à la recherche (Rao et al. 2006).

#### *1.2.1.5 Traitement individuel*

Dans cette partie, nous présentons les différentes stratégies thérapeutiques de prise en charge de la filariose lymphatique au niveau individuel. Les stratégies de lutte contre la maladie au niveau des populations reposant sur des traitements de masse répétés chaque année sont présentées dans la partie 1.3.

Un traitement individuel est préconisé dans les pays où la filariose lymphatique n'est pas endémique comme en Europe et en Amérique du Nord, pour les cas importés. Il existe différents schémas thérapeutiques :

- L'utilisation de doxycycline (200 mg/jour) pendant quatre semaines pour avoir une action macrofilaricide (élimination de 90% des vers adultes) ou six semaines pour avoir une amélioration clinique puis suivie, trois mois plus tard, par une dose unique de DEC (6 mg/kg). Cette stratégie thérapeutique est la seule qui permet actuellement d'éliminer l'infection. Néanmoins, la doxycycline possède plusieurs caractéristiques à prendre en compte : elle est formellement contre-indiquée chez la femme enceinte ou allaitante et chez l'enfant de moins de 8 ans, elle expose à une photosensibilité accrue, pouvant être problématique notamment en Afrique.
- En cas de filariose à *B. malayi*, un traitement par doxycycline (100 mg par jour) pendant 42 jours, suivi par une prise de DEC et d'albendazole est une possibilité.

- Enfin, l'utilisation de DEC (6 mg/kg/jour) pendant deux semaines est indiquée dans la forme du poumon éosinophile.

## 1.2.2 Onchocercose

### *1.2.2.1 Présentation*

L'onchocercose, surnommée « cécité des rivières » est transmise par des simulies, de petits moucherons qui constituent l'hôte intermédiaire du parasite. Ces simulies sont présentes principalement dans les zones humides et se reproduisent dans les cours d'eau rapides. Chaque espèce de simulies possède une répartition géographique spécifique, en relation avec le biotope de sa région. Parmi les différentes espèces de simulies vectrices de l'onchocercose, *Simulium damnosum* est la plus fréquente en Afrique centrale. Les filaires adultes d'*Onchocerca volvulus*, le parasite responsable de l'onchocercose, sont non pathogènes, de grande taille (20 à 70 cm pour les femelles) et vivent dans le derme ou le tissu sous-cutané où elles se regroupent et engendrent l'apparition de nodules, qui sont dus à une réaction du corps de l'hôte qui tente de les isoler. Elles possèdent une longévité importante, de l'ordre de dix ans.

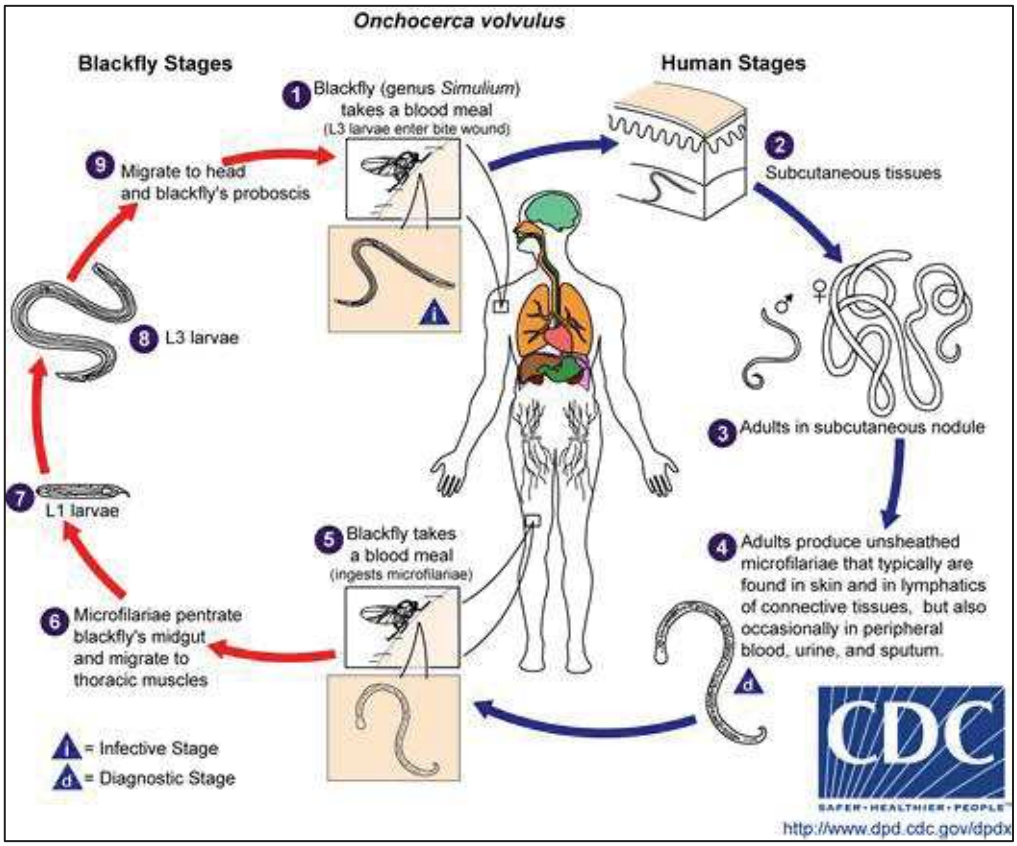



**Figure 3. Cycle parasitaire de l'onchocercose**

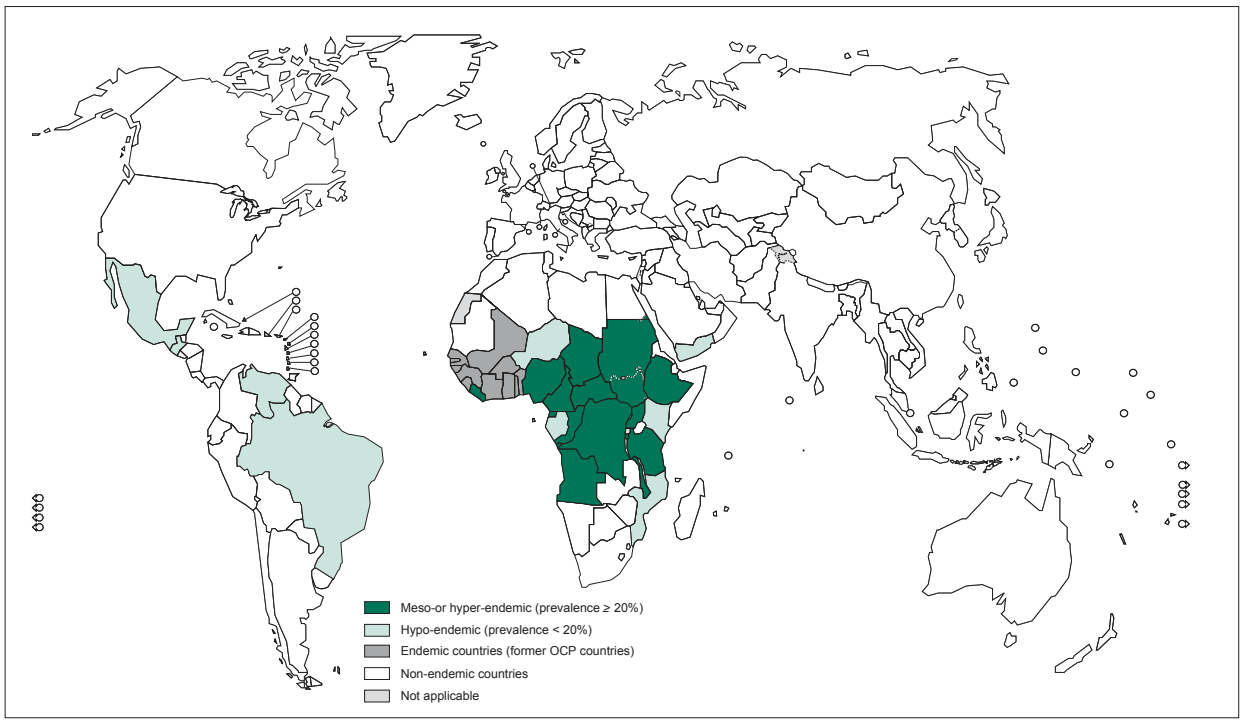
Les microfilaires vivent dans le derme et dans l'œil où elles peuvent provoquer lésions et inflammations. Les microfilaires vivent pendant un à deux ans.

A l'instar de la filariose lymphatique, le parasite responsable de l'onchocercose vit en symbiose avec des bactéries intracellulaires du genre *Wolbachia* qui ont un rôle dans la pathogénicité, la fertilité et la survie du parasite ainsi que dans l'apparition d'effets secondaires post- traitement.

L'Homme est l'unique hôte définitif d'*O. volvulus*. La transmission est induite par le repas de sang du vecteur sur l'hôte définitif (figure 3, source : CDC).

### *1.2.2.2 Épidémiologie*

Avant le lancement des programmes de lutte, il était estimé qu'environ 40 millions de personnes étaient atteintes de l'onchocercose dont 99% vivaient dans 31 pays d'Afrique subsaharienne, avec des niveaux d'endémicité variables (figure 4, source : OMS). En 2020, l'OMS estime qu'environ 218 millions de personnes vivent en zone d'endémie pour l'onchocercose (WHO 2020).






Data Source: World Health Organization
Map Production: Control of Neglected Tropical Diseases (NTD)
World Health Organization

World Health Organization

**Figure 4. Répartition mondiale de l'onchocercose**

Afin de cartographier la répartition de l'onchocercose, différents indicateurs épidémiologiques ont été utilisés.

Initialement, ce sont des indicateurs cliniques qui étaient utilisés. Ainsi, les prévalences de la cécité, des dépigmentations tibiales et des nodules ont été utilisées pour réaliser des évaluations communautaires (Edungbola et al. 1983, 1987; Carme et al. 1993). Une méthode rapide basée sur la palpation des nodules chez environ 50 hommes de plus de 20 ans choisis aléatoirement, a été utilisée pour évaluer le niveau d'endémicité de l'onchocercose dans les communautés (REA: *Rapid Epidemiological Assessment*). Cette méthode a permis de cartographier les communautés selon leurs niveaux d'endémicité dans le but de distinguer les zones où un traitement de masse est prioritaire (prévalence > 40%), souhaitable (prévalence entre 20 et 40%) ou non souhaitable (prévalence < 20%) (Ngoumou et al. 1994). De manière similaire, la méthode de cartographie REMO (*Rapid Epidemiological Mapping of Onchocerciasis*), toujours utilisée actuellement, a permis de visualiser la distribution géographique de l'onchocercose (figure 5, source : *(Zouré et al. 2014))*. Elle est réalisée en 3 étapes successives : la division des pays d'intérêt en fonction de facteurs climatiques et environnementales, la sélection des communautés à étudier et la mesure de la prévalence des nodules (REA).

Deux indicateurs parasitologiques sont également utilisés : l'indice microfilarien, permettant de définir les niveaux d'endémicité : hyperendémie (microfilarodermie supérieure ou égale à 60%, mésoendémie (microfilarodermie entre 35 et 60%) et hypoendémie (moins de 35% de microfilarodermie) (Prost et al. 1979) et le niveau d'endémie communautaire, basé sur la mesure de la charge microfilarienne communautaire (CMFL ; *Community Microfilarial Load*) (Remme et al. 1986). Cet indicateur est plus sensible pour l'évaluation des changements épidémiologiques que la prévalence microfilarienne, notamment dans les zones hyperendémiques. La CMFL correspond à la moyenne géométrique de Williams des charges microfilariennes dermiques individuelles dans la population des plus de 20 ans.

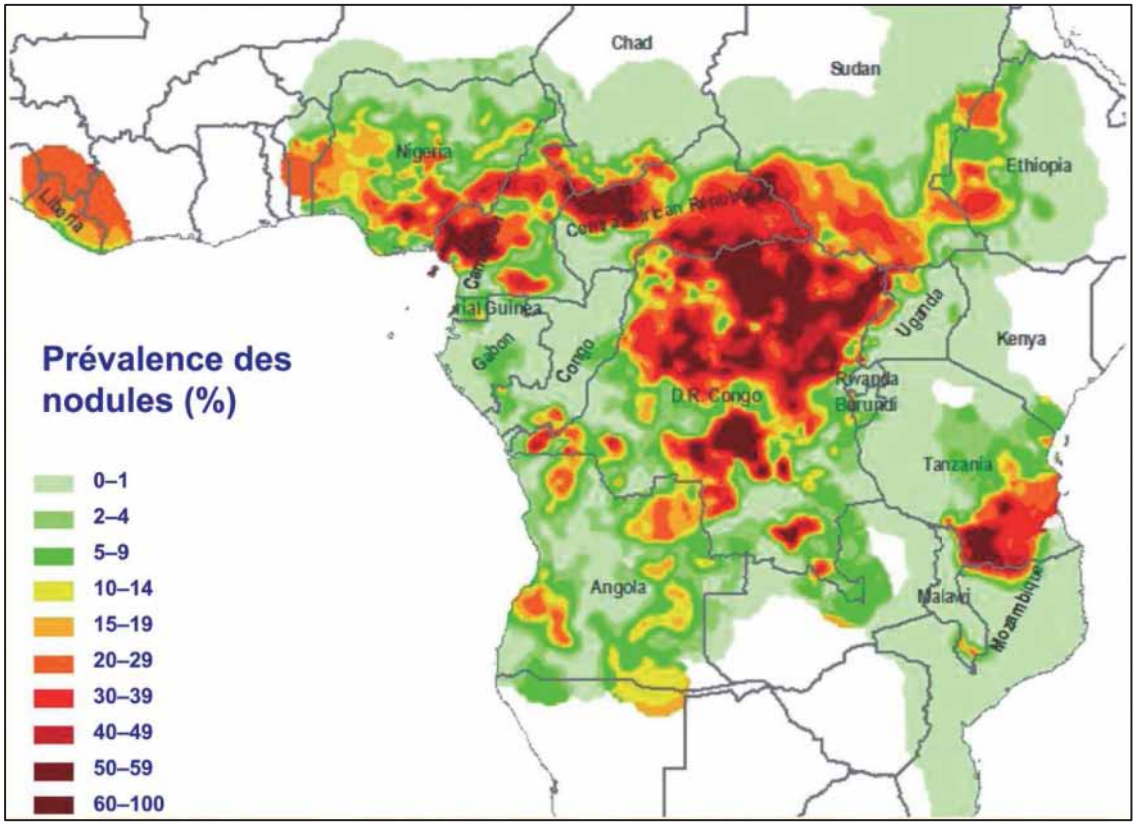



**Figure 5. Cartographie de l'onchocercose en Afrique Centrale par REA et REMO**

### *1.2.2.3 Manifestations cliniques*

## A. Manifestations localisées

Une des manifestations courantes de l'onchocercose est l'apparition de nodules, qui peuvent être sous-cutanés ou profonds et qui sont généralement indolores. Ils sont provoqués par le système immunitaire de l'hôte qui cherchent à isoler les vers adultes. Ces nodules sont, le plus souvent, situés en regard des plans osseux (crêtes iliaques, trochanters, genoux voire crâne).

Comme dans la majorité des infections vermineuses avec migration trans-viscérale, l'onchocercose peut également provoquer des manifestations cutanées : prurit, dermatites papulaires aiguës ou chroniques, dermatites lichénifiées, atrophie cutanée voire dépigmentation cutanée localisée préférentiellement au niveau des crêtes tibiales (Murdoch et al. 1993). Des atteintes oculaires peuvent également survenir. On différencie les atteintes du segment antérieur et celle du segment postérieur de l'œil. Parmi les formes touchant le segment antérieur, on note les iridocyclites (uvéites antérieures) et les kératites avec différents niveaux d'atteinte de la cornée (ponctuée, semi-lunaire, puis sclérosante) pouvant aboutir à une cécité

au stade final de la kératite sclérosante. Parmi les formes touchant le segment postérieur, on note les atrophies optiques et les choriorétinites qui peuvent toutes deux aboutir à une cécité.

L'onchocercose a également été associé à un risque de mortalité plus élevé chez les personnes atteintes par des charges microfilariennes importantes mais ne présentant pas de symptômes oculaires (Little et al. 2004).

L'onchocercose est une maladie aux conséquences socio-économiques importantes par les symptômes qu'elles entrainent. Les manifestations oculaires entrainent un handicap et une surmortalité (Pion et al. 2002). Les manifestations cutanées comme le prurit perturbent la qualité de vie et de travail des personnes infectées et les modifications cutanées qu'elles entrainent peut provoquer des stigmatisations des malades. On estime à environ 1,5 millions de DALYs (*disability-adjusted life years* - années de vie perdues ajustées sur l'incapacité due à la maladie) le poids de l'onchocercose (Basáñez et al. 2008).

## B. Manifestations systémiques

L'onchocercose engendre également des symptômes systémiques comme le retard staturopondéral, le nanisme infantile et le syndrome du hochement de tête (*nodding syndrome*), une forme d'épilepsie atonique touchant essentiellement les enfants et qui associe un retard de croissance, une atrophie cérébrale et un hochement de tête caractéristique ayant donné le nom au syndrome.

## C. Focus sur l'épilepsie

L'association possible entre épilepsie et onchocercose a longtemps été sujet à débat dans la communauté scientifique. Cette association a été décrite pour la première fois en 1938 au Mexique (Casis Sacre 1938). Depuis 2002, plusieurs études de types cas-témoins, transversales, ou encore écologiques, ont décrit cette association en Afrique (Kaiser et al. 1996; Dozie et al. 2006; Druet-Cabanac et al. 2004; Boussinesq et al. 2002; Kaiser et al. 2008, 2013; Pion et al. 2009). En 2017, une étude de cohorte rétrospective réalisée dans la vallée du Mbam au Cameroun a étudié cette association. Elle a permis de confirmer, pour la première fois, que l'incidence de l'épilepsie chez les adultes était positivement corrélée à l'intensité de l'infection onchocerquienne dont ils étaient atteints pendant leur jeunesse. Cela a permis de valider deux des critères de causalité de Bradford Hill : la relation temporelle et le gradient d'association entre cause et effet (Chesnais et al. 2018).

En 2019, un autre critère de Bradford Hill a pu être évalué : la reproductibilité. Pour cela, une étude similaire mais dans un site différent, le département de la Lékié au Cameroun a été réalisé. Cette étude a permis de valider définitivement ce critère permettant ainsi de conforter l'existence d'une relation causale entre l'infection par *O. volvulus* et l'épilepsie ***(article disponible en partie Annexes)***.

Néanmoins, il reste encore des critères qui nécessitent validation : la plausibilité biologique et la preuve animale. En effet, malgré l'existence d'hypothèses, l'explication physiopathologique reste à démontrer. Parmi les hypothèses pouvant expliquer cette association, la plus plausible semble être la pénétration directe des microfilaires dans le système nerveux central (Chesnais et al. 2018). Des mécanismes indirects auto-immuns mettant en jeu des mimétismes moléculaires pourraient également être une explication (Johnson et al. 2017). Cependant, la présence d'une forte relation dose-réponse supporte plutôt l'hypothèse d'un mécanisme direct. Il serait pertinent de développer un modèle animal afin de pouvoir tester plus précisément ces hypothèses mécanistiques.

#### *1.2.2.4 Méthodes diagnostiques*

##### A. Diagnostic clinique et diagnostic direct

Un diagnostic reposant sur l'examen clinique du patient est possible dans le cadre de l'onchocercose. Les nodules, la dépigmentation des crêtes tibiales et la présence de microfilaires dans la chambre antérieure de l'œil sont tous les trois des signes pathognomoniques de la maladie. L'examen ophtalmologique à l'aide d'une lampe à fente permet de visualiser les microfilaires vivantes et mobiles dans la chambre antérieure de l'œil.

Le diagnostic de référence reste cependant le diagnostic parasitologique. Il repose sur la visualisation directe des microfilaires sur une biopsie cutanée exsangue (BCE). Cette biopsie est réalisée préférentiellement sur les crêtes iliaques grâce à des pinces cornéosclérales de type Holth ou Walzer. Les prélèvements sont ensuite placés pendant 30 minutes à 24 heures à température ambiante dans un puit d'une plaque de micro-titration contenant du sérum physiologique. Le surnageant est ensuite prélevé, étalé sur une lame et les microfilaires sont alors directement comptées au microscope. La moyenne arithmétique de deux biopsies est calculée, et le résultat est exprimé en nombre de microfilaires par biopsie. L'allongement du temps d'incubation du *snip* dans le sérum physiologique a pour effet une augmentation de la sensibilité

du diagnostic (Collins et al. 1980). Le diagnostic par BCE fut longtemps considéré comme ayant une spécificité proche de 100% malgré la confusion possible avec d'autres filaires du genre *Mansonella* dans les prélèvements (Moraes 1976; Fischer et al. 1998). En 2019 et en 2021, deux études mettent en évidence l'existence d'une relation entre haute microfilarémie à *Loa loa* et fausse-positivité des *skin snip* pour l'onchocercose, principalement due à leur similarité morphologique. En effet, les microfilaires à *L. loa* peuvent émerger des artérioles et veinules dermiques lors du prélèvement du *skin snip* et être confondus avec les microfilaires d'*O. volvulus* lors de la lecture microscopique (Nana-Djeunga et al. 2019; Niamsi-Emalio et al. 2021). Il est également possible de réaliser un diagnostic parasitologique de l'onchocercose par coloration des biopsies cutanées au May-Grünewald Giemsa voire par la réalisation de coupes histologiques cutanées colorées à l'hématoxyline et à l'éosine mais ces pratiques sont peu fréquentes en routine.

Il existe des bandelettes capables de détecter des antigènes spécifiques dans les fluides biologiques (urines, larmes et sang) et reposant sur des techniques d'ELISA ou de Western Blot. Ces tests ne sont pas utilisés en routine (Haute Autorité de Santé 2018).

Enfin, une PCR ciblant une séquence d'ADN répétitive O-150 dans le génome d'*O. volvlulu*s permettrait un diagnostic sensible et spécifique (Zimmerman et al. 1994). La principale limite de cette PCR est qu'elle nécessite un prélèvement cutané. Cette technique est encore limitée au domaine de la recherche.

## B. Diagnostic indirect

Historiquement, l'utilisation d'un test d'épreuve appelé test de Mazzotti était réalisé pour diagnostiquer l'onchocercose, il reposait sur l'administration par voie orale d'une dose infra-curative (50 mg) de DEC (Mazzotti 1948). Si le patient était infecté par l'onchocercose, une réaction cutanée de type rash papulaire avec prurit apparaissait. Ce test de Mazzotti est désormais désuet du fait de fortes réactions indésirables possibles de type fièvre, malaise, hypotension, œdèmes, jusqu'au choc, ainsi que l'apparition voire l'aggravation de lésions oculaires en cas de microfilaires d'*O. volvulus* présentes dans la chambre antérieure ou la vitrée postérieure. Une technique alternative d'intérêt reposant sur la même méthode a été élaborée dans les années 1980 (Toè et al. 2000). Ce test utilise également la capacité microfilaricide de la DEC mais seulement au niveau local par l'application d'un patch contenant de la DEC.

La détection d'anticorps spécifique de l'onchocercose peut permettre de diagnostiquer une exposition à l'infection mais ne permet pas de déterminer si l'infection est active ou ancienne. Deux catégories de tests existent :

La détection d'antigènes pan-filariens (*Dirofilaria immitis*, *Dipetalonema vitae*…) : elle peut permettre de diagnostiquer de façon non spécifique un nombre important d'infections filariennes dont celle à *W. bancrofti*, *Brugia* spp., *L. loa*, *Mansonella* spp. et *O. volvulus.* Il n'est pas possible de faire un diagnostic d'espèce. Cette détection peut reposer sur 3 méthodes : la réaction de précipitation en gélose, l'ELISA et l'immunofluorescence indirecte. Ce test n'est pas utilisé en routine dans les campagnes de lutte contre l'onchocercose (Haute Autorité de Santé 2018), mais trouve sa place dans les structures hospitalières et les services de médecine du voyage.

Une technique ELISA permet une détection plus spécifique des IgG4 dirigés contre l'Ag recombinant Ov16 de l'onchocercose (Lobos et al. 1990, 1991). Il est utilisé dans les programmes d'élimination de l'onchocercose. Une autre technique basée sur une réaction d'immunoprécipitation-luminescence *(Luciferase Immunoprecipitation Systems ; LIPS)* pourrait également permettre la détection d'anticorps spécifiques de l'onchocercose. Ces tests sérologiques sont prometteurs, ils permettent, par exemple, l'identification de sujets en phase pré-patente, avant la présence des microfilaires à partir d'un prélèvement de sang au bout du doigt (Golden et al. 2013). Ils pourraient donc être d'une grande aide dans la détection des recrudescences d'infection dans les populations à risques. Cependant, la cinétique des anticorps de l'onchocercose n'est pas encore complétement élucidée et les performances diagnostiques de ces tests sont encore sujets à débat. La détection des anticorps dirigés contre les antigènes Ov16 est également possible par l'utilisation d'une méthode rapide appelée « cassettes » qui sont rapides et simples à utiliser, appropriées à une utilisation de terrain et peu invasives à partir d'un échantillon de sang capillaire.

#### *1.2.2.5 Traitement individuel*

L'ivermectine est la molécule de référence pour la prise en charge thérapeutique de l'onchocercose. Ce médicament est connu pour avoir deux activités principales : un effet microfilaricide et un effet embryostatique (Basáñez et al. 2008). Au-delà de ces deux mécanismes, l'ivermectine a également une faible action macrofilaricide. Cela implique qu'il est

nécessaire de répéter les traitements pour maintenir une faible densité de microfilaires cutanés et éviter ainsi toute manifestation clinique (Walker et al. 2017). En outre, l'ivermectine a été peu étudiée pour un éventuel effet prophylactique potentiel (c'est-à-dire son effet sur les stades larvaires et les femelles juvéniles d'*O. volvulus*). L'ivermectine est administrée en prise unique à la dose de 150 µg/kg. Elle permet une baisse rapide des densités microfilariennes au niveau dermique pendant généralement deux à trois mois (en fonction de l'effet embryostatique obtenu). Une prise de corticoïdes par voie orale pendant trois à quatre jours, concomitante à la prise d'ivermectine peut être envisagée chez certains sujets.

Seule la doxycycline possède une forte action macrofilaricide sur l'onchocercose (Walker et al. 2015). Par action sur les *Wolbachia* symbiotiques, elle permet la destruction d'environ 70 % des vers adultes. Au niveau individuel, la prise en charge repose donc sur la doxycycline, avec une administration de 200 mg par jour pendant six semaines. Les nodules sous-cutanés peuvent être retirés par exérèse chirurgicale.

## 1.2.3 Loase

### *1.2.3.1 Présentation*

La loase est une filariose qui, contrairement à l'onchocercose, est dite « à microfilaires sanguicoles » car les embryons du ver, les microfilaires, circulent dans le sang. Elle est transmise par des taons du genre *Chryspos* notamment *C. silacea* et *C. dimidiata*. On estime à environ dix millions le nombre de personnes infectées par le parasite *Loa loa* (Zouré et al. 2011) et à 14 millions le nombre d'individus vivant en zone à haut risque d'infection par *L. loa*. Dans ces zones, la prévalence de l'infection y est d'environ 60% et celle du passage sous-conjonctival de l'œil est estimé à environ 40%. On estime également que 15,2 millions d'individus vivent dans des zones à risque modérée où 20 à 40% des individus reportent avoir déjà eu un passage sous-conjonctival du ver (Whittaker et al. 2018).

La loase est géographiquement bien limitée car elle n'est présente qu'en Afrique, et majoritairement dans le bloc forestier d'Afrique centrale. Les vers adultes sont plus grands que ceux de la filariose lymphatique (4 à 7 cm pour les femelles), ils vivent dans la peau et dans les fascias intermusculaires. Ils sont capables de migrer en direction des tissus de l'œil. Leur longévité est extrêmement importante puisqu'ils peuvent vivre jusqu'à 20 ans.

Il existe une périodicité diurne de la microfilarémie due au même phénomène de coévolution entre le parasite et le vecteur que la filariose lymphatique, qui conduit à une synchronisation entre la période d'activité vectorielle et la périodicité des microfilaires dans le sang périphérique.

Il existe plusieurs hôtes définitifs : l'Homme et plusieurs espèces de singes (drills, babouins…) mais cependant chaque type d'hôte est infecté par une souche différente (figure 6, source : CDC). L'hôte intermédiaire est le *Chrysops* femelle.

La loase n'est pas, à l'heure actuelle, considérée comme une maladie tropicale négligée car sa symptomatologie est considérée comme bénigne et que sa répartition est restreinte à l'Afrique centrale.

Une des caractéristiques de la loase, contrairement aux autres helminthiases, est sa capacité à engendrer une forte densité en microfilaires par ml de sang chez les individus

parasités, parfois jusqu'à plus de 300.000 microfilaires par ml de sang, et qui reste stable dans le temps (Noireau and Pichon 1992; Pion et al. 2004; Garcia et al. 1995; Pion et al. 2019) avec une certaine prédisposition génétique à avoir une même densité microfilarienne tout au long de sa vie (Garcia et al. 2003; Eyebe et al. 2018).

Contrairement aux deux autres filarioses présentées, la loase ne possède pas de bactérie symbiotique *Wolbachia*, ce qui écarte l'efficacité possible des antibiotiques sur ce parasite.

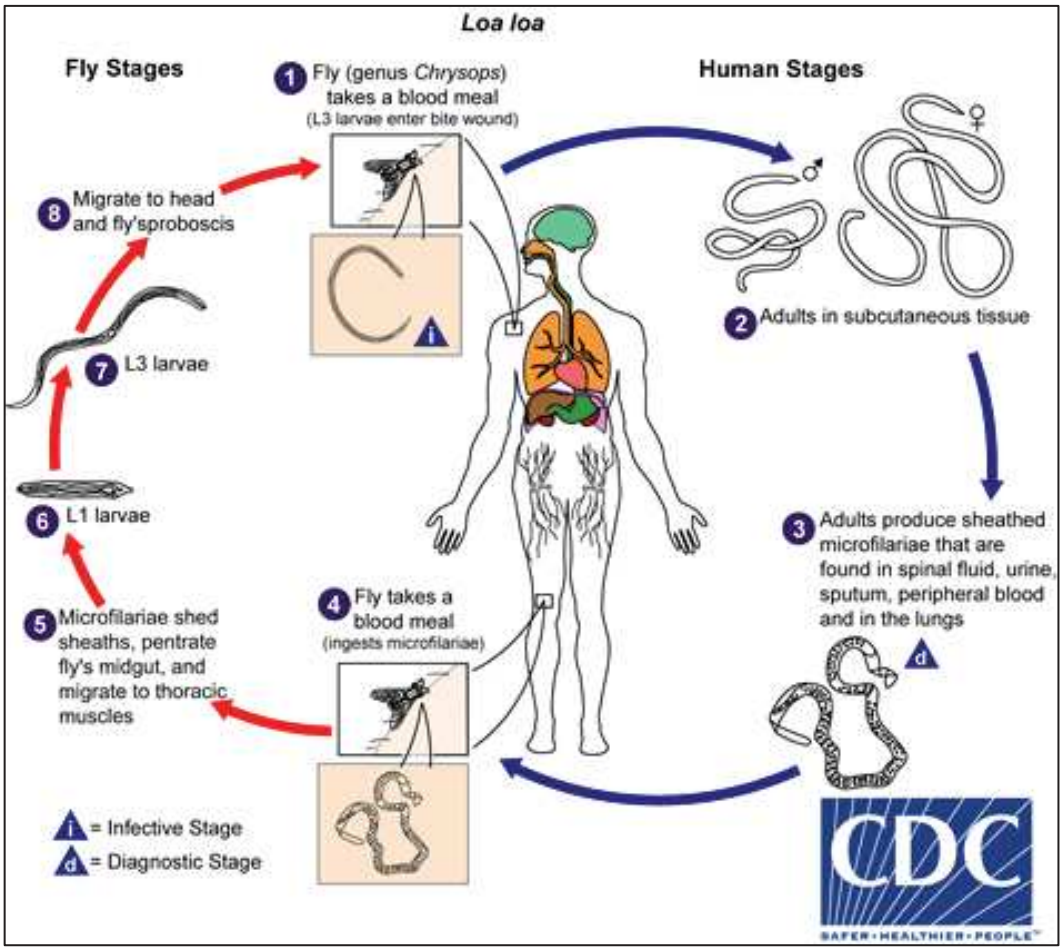



**Figure 6. Cycle parasitaire de la loase**

### *1.2.3.2 Épidémiologie*

La loase a une distribution géographique limitée à l'Afrique centrale (figure 7, source : (Zouré et al. 2011)). Le programme africain de lutte contre l'onchocercose a établi une carte de la loase sur 11 pays d'Afrique centrale grâce à une procédure d'évaluation rapide pour la loase (RAPLOA pour *rapid assessment procedure for loiasis*). Cette procédure (Zouré et al. 2011) est basée sur l'administration de questionnaires aux habitants de différents villages. Plus de 4800 villages ont été enquêtés pour un total d'environ 350 000 personnes interrogées. Le

questionnaire utilise l'antécédent de passage sous-conjonctival du ver adulte pour évaluer la présence de la loase dans les villages. Il en a résulté l'identification de deux blocs à haute prévalence : un bloc comprenant la partie Sud et Est du Cameroun, l'Ouest de la République Centrafricaine, la Guinée équatoriale, le Gabon, la partie Ouest de la République du Congo et un bloc comprenant la partie Nord et Nord Est de la République Démocratique du Congo et le Soudan du Sud.

En plus de la méthode rapide d'identification du niveau d'endémie à la loase basée sur questionnaires, la densité microfilarémique est également utilisée pour définir le niveau d'endémie de la loase dans une région ; elle est cependant compliquée à utiliser comme indicateur épidémiologique car une partie de la population infectée reste amicrofilarémique (Garcia et al. 2003) – ce qui sous-estime la prévalence de la loase dans certaines régions.

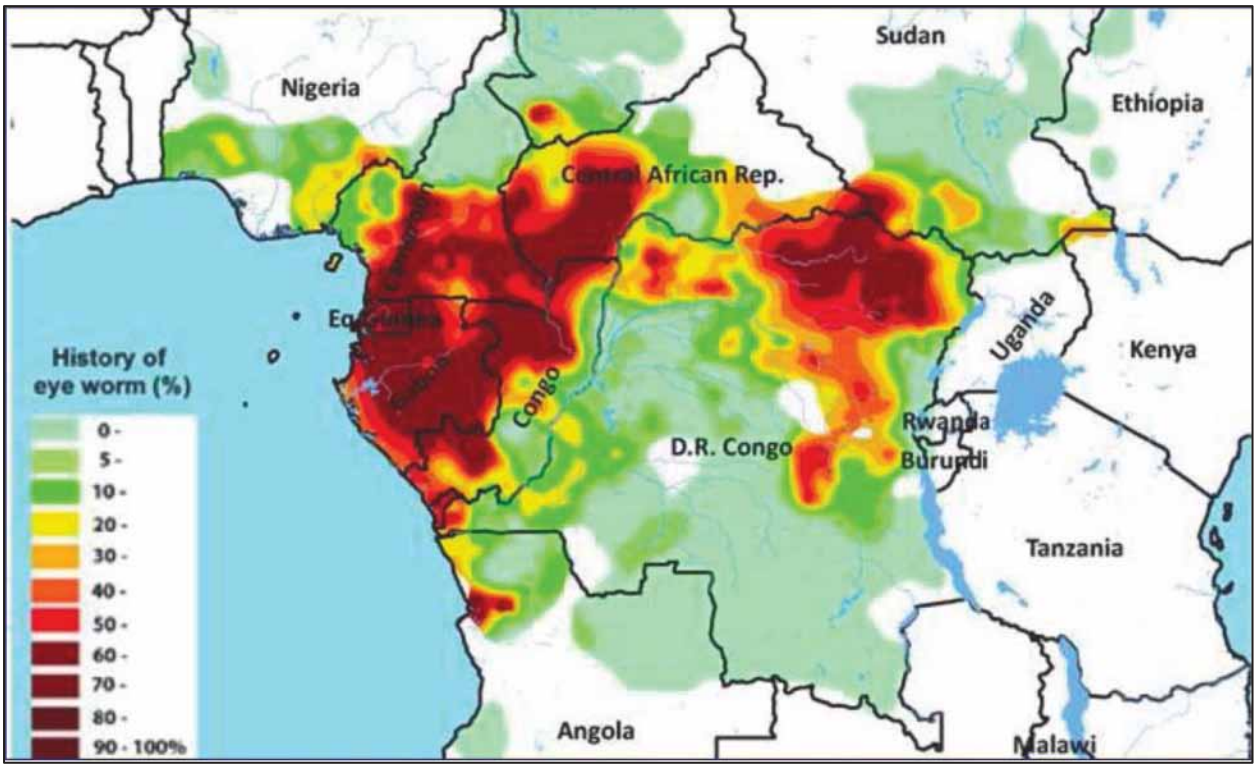



**Figure 7. Répartition géographique de la loase selon RAPLOA**

### *1.2.3.3 Manifestations cliniques*

Dans la plupart des cas, la symptomatologie de la loase est bénigne voire asymptomatique mais cependant non négligeable car elle fait partie des causes fréquentes de consultation en zone d'endémie (Richard et al. 1988; Boulesteix and Carme 1990).

Les signes cliniques peuvent apparaitre dès le 5ème mois post-infection et jusqu'à 13 ans après. Les symptômes caractéristiques de la pathologie (Boussinesq et al. 2003) sont :

- Un prurit intense et très fréquent
- Un œdème de Calabar qui est un œdème du tissu sous-cutané provoqué par la migration des larves depuis le point d'inoculation du vecteur (Basáñez et al. 2008) ; et qui pourrait être de nature immuno-allergique (T. Nutman et al. 1988). Une des complications rares est la compression du nerf périphérique en regard de l'articulation pouvant entraîner secondairement des problèmes locomoteurs (Padgett and Jacobsen 2008).
- La migration transitoire du ver mature au niveau de la sous-conjonctive ou dans la sclère de l'œil ; provoquant douleurs intenses, inflammations sévères voire, dans quelques cas anecdotiques, une cécité. Cette migration donne le nom de maladie de « ver de l'œil » à la loase.

La loase provoque également une hyperéosinophilie importante et persistante. A ces signes cliniques « assez bénins », il faut rajouter les complications rares mais graves comme (Boussinesq 2007) :

- Les atteintes rénales (protéinurie, hématurie, syndrome néphrotique, glomérulopathies…)
- Les manifestations neurologiques spontanées peu documentées due à la difficulté d'établir un lien causal chez des patients poly-infestés. Elles semblent cependant exceptionnelles et graves.
- L'endocardite fibroplastique éosinophilique due à l'hyperéosinophilie persistante.
- Les complications oculaires (hémorragies rétiniennes…)
- Les arthropathies (épanchements avec présence de microfilaires dans le liquide synovial)

Si l'ensemble de ces complications sévères ou pouvant être appelées « atypiques » sont uniquement connues grâce à la publication sous forme de case-reports, une méta-analyse récente de ces publications a permis de montrer que ces complications « atypiques » augmentent principalement chez les individus présentant de fortes densités microfilariennes à *L. loa* (Buell et al. 2019). De plus, une étude rétrospective réalisée dans la Région de l'Est du Cameroun a montré une excès de mortalité chez les individus fortement parasités, avec près d'un décès sur dix qui aurait pu être évité en l'absence d'infection à *L. loa* dans la zone d'étude (Chesnais et al. 2016). Pour toutes ces raisons, la loase ne devrait pas être considéré comme une

maladie bénigne mais comme une maladie tropicale négligée nécessitant la mise en place de programmes de recherche et de lutte.

#### *1.2.3.4 Méthodes diagnostiques*

### A. Diagnostic clinique et diagnostic direct

Le diagnostic par examen clinique dans le cas de la loase peut reposer sur l'examen de l'œil et du passage sous-conjonctival du ver et sur la présence de l'œdème caractéristique de l'infection : l'œdème de Calabar.

Le diagnostic parasitologique repose sur une technique de goutte épaisse calibrée ; du fait de la périodicité diurne de *L. loa*, il est recommandé de réaliser ce diagnostic entre 10 heures et 16 heures. Il consiste à prélever environ 50 µL de sang qui est déshémoglobinisé et coloré au Giemsa sous 24 heures puis examiné au microscope. En cas de densités très faibles, il est possible de réaliser une leucoconcentration afin d'augmenter la sensibilité de l'examen. Un des problèmes de cette technique est la « loase occulte », il s'agit d'une prédisposition génétique qui touche 40% de la population, les personnes infectées ne présentent pas des microfilaires sanguines et sont donc considérés comme des faux négatifs. Une technique diagnostique plus actuelle, appelée LoaScope (D'Ambrosio et al. 2015) permet le comptage automatique des microfilaires par un outil informatique à partir d'un capillaire sanguin introduit dans un dispositif associé à un smartphone. Cette technique repose sur la visualisation des microfilaires repoussant les hématies grâce à un système de grossissement. Cinq vidéos de 3 secondes chacune permettent grâce à un algorithme installé dans le smartphone d'estimer le nombre de microfilaires et d'afficher la densité en microfilaires par millilitre. Les principaux avantages du LoaScope sont la rapidité, le résultat est obtenu en moins d'une minute et les très bonnes performances du dispositif. Il présente cependant les mêmes limites que la GEC.

Actuellement, il n'existe aucun test basé sur la détection d'antigènes de loase. Il existe deux tests permettant la détection spécifique de l'ADN de loase. Le premier repose sur une PCR quantitative (Fink et al. 2011) et le deuxième sur la technique *Loop-mediated isothermal amplification (LAMP)*, il s'agit d'un technique colorimétrique semi-quantitative plus facilement applicable car nécessitant moins de matériel mais utilisée uniquement dans le cadre de la recherche (Touré et al. 1997; Toure et al. 1998; Fink et al. 2011; Drame et al. 2014)

## B. Diagnostic indirect

La détection d'anticorps circulants spécifiques permet de diagnostiquer la loase, même occulte. Mais, cette technique ne permet pas de distinguer une infection passée d'une infection présente. Dès lors, cela limite l'intérêt de la sérologie en zone endémique. Ce test a été utilisé au niveau communautaire pour permettre de cartographier la loase. L'intérêt de la sérologie serait de poser un diagnostic d'exclusion en cas de négativité (haute valeur prédictive négative - VPN). Deux techniques de sérologies existent : la recherche d'anticorps pan-filariens non spécifiques de la loase (techniques IELP, IFI et ELISA) et la recherche des anticorps IgG4 couplées à un antigène recombinant spécifique de *L. loa*. Cette dernière a pu être mis en évidence par l'utilisation de sérums de singes infectés expérimentalement par *L. loa*, ayant permis d'identifier l'antigène LI-SXP-1. Cette technique présentait initialement une sensibilité de 56% et une spécificité de 98% (Klion et al. 2003). Par la suite, l'utilisation d'une technique d'immunoprécipitation de l'IgG Luciférase et de bioluminescence (technique LIPS) a permis d'améliorer la sensibilité à 67% et la spécificité à 94% (Burbelo et al. 2008). Enfin, Pedram et ses collègues ont mis au point un test de diagnostic rapide à flux latéral basé sur l'antigène recombinant Ll-SXP-1, avec une sensibilité encore améliorée (94 %) et une spécificité acceptable (82 %-100 % selon le panel de contrôle testé) (Pedram et al. 2017). Un tel test rapide pourrait alors trouver sa place dans les services des médecine du voyage hors zone endémique (Gobbi et al. 2020).

### *1.2.3.5 Traitements*

En théorie, il existe trois médicaments utilisables contre la loase : la DEC, l'ivermectine et l'albendazole. Le seul médicament ayant un effet macrofilaricide est la DEC. Pour un traitement individuel, le protocole clinique est basé sur la mesure systématique de la microfilarémie et l'utilisation d'une stratégie permettant une diminution progressive des charges microfilariennes de manière à prévenir les effets secondaires graves possibles.

On classifie les charges microfilariennes en quatre catégories de risque : <2000 mf/ml ; entre 2000 et 8000 mf/ml, on a un risque d'effet secondaire grave neurologique post-DEC ; entre 8000 et 30000, on a un risque d'effet secondaire grave neurologique post-DEC et un risque effet secondaire grave non neurologique post-ivermectine ; au-delà de 30.000 mf/ml, on a un risque d'effets secondaires neurologiques graves post-DEC et post-ivermectine.

En 2012, Boussinesq a proposé des stratégies de traitement individuelles en fonction des catégories de risque des densités microfilariennes (Boussinesq 2012), résumées en Tableau 1.

| Densité microfilarémique | Molécules proposées | Durée de traitement | Posologie | Remarques |
|---|---|---|---|---|
| Amicrofilarémique | Diéthylcarbamazine | 3 à 4 semaines | Départ à 50 mg/jour puis x2 tous les jours jusqu'à 400 mg/jour | ▪ En milieu hospitalier<br>▪ Utilisation de corticostéroïdes et antihistaminiques en cas d'effets secondaires<br>▪ Plusieurs rounds de traitement peuvent être nécessaires |
| < 2000 mf/ml | Diéthylcarbamazine | 3 à 4 semaines | 3 à 6 mg/kg/jour | |
| | Albendazole | 3 à 4 semaines | 200 mg 2 x par jour | ▪ Si le patient ne répond pas à la DEC |
| 2000 – 8000 mf/ml | Ivermectine | Dose unique | 150 µg/kg | ▪ L'objectif est d'atteindre 2000 mf/ml. Une fois atteint, se référer à la catégorie <2000 mf/ml<br>▪ Ce prétraitement peut être répété tous les 1 à 3 mois |
| 8000 – 30 000 mf/ml | Ivermectine | Dose unique | 150 µg/kg | - Sous surveillance médicale étroite |
| | Albendazole<br>*Puis* Ivermectine | 3 semaines<br>Dose unique | 200 mg 2 x par jour<br>150 µg/kg | |
| > 30 000 mf/ml | Albendazole | Protocole de traitement à définir et difficilement mis en place en pratique | | |
| | Aphérèse | 3 sessions | | - Cher et difficilement indicable en cas de symptomatologie légère |

**Tableau 1. Algorithme décisionnel de prise en charge de la loase au niveau individuel**

Parmi les contre-indications de la DEC, on note l'onchocercose associée, l'altération de l'état général et la grossesse. Les stratégies thérapeutiques sont donc extrêmement limitées et on comprend bien que les zones de coendémie à l'onchocercose et à la loase posent donc un réel problème de prise en charge efficace et sûre. On note, également, qu'aucune directive de la part de l'OMS ne préconise le traitement de la loase.

## 1.3 Programmes de santé publique contre les filarioses

### 1.3.1 Historique des programmes de lutte

#### *1.3.1.1 Programmes de lutte contre l'onchocercose*

En 1974, l'*Onchocerciasis Control Programme in West Africa* (OCP) est lancé grâce à la collaboration entre l'OMS, la Banque Mondiale, le Programme des Nations Unis pour le développement et l'Organisation des Nations Unies pour l'alimentation et l'agriculture. L'OCP cible 11 pays d'Afrique de l'Ouest et 30 millions de personnes. Jusqu'en 1987, la stratégie de lutte contre l'onchocercose était basée sur la lutte anti-vectorielle : épandages d'insecticides par voie aérienne sur les lieux de reproduction des simulies. Le but de ce programme est d'interrompre la transmission pendant au moins 13 ans ; correspondant à la longévité des vers adultes. En 1988, le programme a été évalué : il a permis une diminution des CMFL et de la prévalence des microfilaires. Cependant, ces diminutions n'ont pas duré puisqu'une évaluation en 2002 a montré que ces deux indicateurs étaient repartis à la hausse dans certaines régions (Organisation mondiale de la Santé et al. 2002).

Le bilan de l'OCP estime qu'il a permis d'éviter environ 200 000 cas de cécités, 40 millions de personnes auraient été protégées du risque de lésions oculaires dues à l'onchocercose. Il reste un des grands succès de l'OMS. Cependant, l'OCP a été assez couteux (un peu moins d'un milliard de dollars). C'est en 1987, grâce à la découverte par S. Omura, W.C. Campbell & Tu Youyou d'un nouveau médicament filaricide ; l'ivermectine, que la stratégie lutte a évolué.

L'ivermectine, par son action sur les canaux chlore dépendant du glutamate présents dans les muscles et le pharynx des vers, va avoir principalement un effet microfilaricide et embryostatique mais n'aura que peu d'impact sur les vers adultes. La longévité des vers adultes étant élevée, l'ivermectine doit être administrée de manière répétée pour permettre l'élimination du parasite chez un individu (Coffeng, Stolk, Hoerauf, et al. 2014). Il est estimé que 14 jours après une prise unique, la réduction de la densité microfilarienne est de 99% (Basáñez et al. 2008). En 1987, l'ivermectine (Mectizan®) obtient son autorisation de mise sur le marché (AMM) et un programme de donation du Mectizan® par la société Merck&Co® est lancé. Entre

1990 et 1995, des programmes de lutte nationaux sont lancés. Cependant, il est estimé que seulement 15% de la population à risque est traitée. C'est pour cela qu'un deuxième programme est lancée en 1995 : l'*African Programme for Onchocerciasis Control* (APOC).

L'APOC a pour objectif l'éliminer l'onchocercose comme problème de santé publique dans 19 pays situés hors de l'aire du premier programme, l'OCP. La stratégie de lutte est le traitement de masse des populations à risque par l'ivermectine sous directives communautaires appelée Traitement par Ivermectine sous Directives Communautaires (TIDC). Il a donc été lancée 108 TIDC dans 15 pays différents d'Afrique centrale pour une population impactée de plus de 100 millions de personnes.

Ce programme a l'avantage d'être beaucoup moins coûteux que l'OCP (environ 200 millions de dollars). De nouveaux défis ont émergés par la mise en application de ce programme :

- Pendant combien de temps faut-il traiter les populations ?
- Comment assurer la suite de la prise en charge lorsque le financement de l'APOC prend fin ?
- Comment assurer la couverture de traitements dans des zones de conflit ?
- Existe-t-il un phénomène de résistance qui émerge par la prise répétée d'ivermectine ?
- Comment gérer les effets secondaires de l'ivermectine dans une situation de traitement de masse ?

L'un des défis principaux émergeant est apparu en 1991 avec les premiers cas d'encéphalopathies parfois mortels en post-prise d'ivermectine au Sud du Cameroun (Carme et al. 1991; Anonymous 1991; Remme 1995; Ducorps et al. 1995; Chippaux et al. 1996). Le lien avec la coinfection à *L. loa* est rapidement suspecté, et en 1995, une étude a permis de démontrer le rôle joué par la loase dans l'apparition de ces effets secondaires graves (Gardon, Gardon-Wendel, Demanga-Ngangue, et al. 1997), avec la démonstration d'une relation dose-effet qui augmente le risque. Cependant, il n'a jamais été observé de tels effets secondaires graves imputable à *Loa loa* en dessous de 50 000 mf/mL, et selon un principe de précaution, un seuil à 30 000 mf/mL a été choisi pour délimiter la densité microfilarienne à *L. loa* au-delà duquel le risque d'effets secondaires graves post-ivermectine peut exister. Ces effets secondaires graves sont caractérisés par un tableau clinique particulier : les premiers symptômes (asthénie, arthralgies, céphalées…)

apparaissent dans les 24 heures suivant la prise d'ivermectine. Vingt-quatre à 48 heures après, des hémorragies de la conjonctive palpébrale peuvent apparaître et constituent un bon signe permettant d'identifier les personnes susceptibles de développer un effet secondaire grave. Ce tableau clinique se complique ensuite rapidement (sous 48-72 heures) par une aphasie, des troubles de la conscience, une incontinence et évolue vers un coma avec divers signes neurologiques. La coendémicité à la loase est donc un frein majeur dans le traitement des zones d'endémies à l'onchocercose et notamment dans les zones fortement infectées par la loase et hypoendémiques pour l'onchocercose où la balance bénéfice/risque d'un traitement de masse n'est pas acceptable.

En 2004, l'OMS émet des recommandations pour le traitement de l'onchocercose à l'ivermectine dans des régions coendémiques à l'onchocercose et la loase, en y indiquant que pour ces zones, seuls les zones méso- et hyperendémiques pour l'onchocercose sont admissibles au traitement de masse par ivermectine seul, administré tous les ans à toute la population de plus de cinq ans inclus.

En 2012, les objectifs de lutte ont changé en passant d'un objectif de contrôle de l'infection (élimination de la maladie en tant que problème de santé publique) à un objectif d'élimination d'ici 2025 de l'infection suite à la démonstration que l'onchocercose pouvait être éliminée par les TIDC (Diawara et al. 2009; Traore et al. 2012; Tekle et al. 2016). Dès lors, il devient impératif de trouver une stratégie afin d'étendre le traitement dans les zones hypoendémiques et d'accélérer les efforts dans les zones méso- et hyperendémiques.

En 2015, l'APOC prend fin et est succédé en mai 2016 par un programme plus global, l'*Expanded Special Project for Elimination of Neglected Tropical Diseases* (ESPEN) qui, avec des moyens moins importants devra lutter contre l'onchocercose et d'autres MTN (filariose lymphatique, schistosomiases, géohelminthiases, trachome). Cette transformation rentre dans le cadre d'une volonté de rationalisation des coûts entre les différents programmes, et de rendre plus efficient l'ensemble des programmes de lutte. L'objectif d'éliminer l'onchocercose d'ici 2025 est donc fortement compromis sans la mise en place de stratégies de traitements alternatives.

#### *1.3.1.2 Programme de lutte contre la filariose lymphatique*

Le *Global Programme to Eliminate Lymphatic Filariasis* (*GPELF*) a été créé en 2000 sous l'impulsion de l'OMS ayant donné, en 1997, l'objectif d'éliminer la filariose lymphatique contre

problème de santé publique en 2020. Les objectifs du GPELF sont d'interrompre la transmission du parasite en réduisant les charges microfilariennes dans les communautés endémiques et de prendre en charge individuellement les manifestations cliniques de la filariose lymphatique.

L'objectif d'éliminer – voire d'éradiquer – l'infection était considéré initialement comme envisageable d'ici 2020 puisque :

- Les médicaments utilisés sont très efficaces sur la microfilarémie,
- Les vers adultes ont une durée de vie relativement courte, ce qui a un impact positif sur la durée des programmes de distribution massive de médicaments
- L'utilisation de la moustiquaire imprégnée peut faciliter l'interruption de la transmission.

Les médicaments utilisés dans les campagnes de distributions massives annuelles de médicaments en Afrique Centrale dépendent de l'endémicité de l'onchocercose :

- Dans les pays où l'onchocercose est endémique, l'association ivermectine (200 à 400 µg/kg) et albendazole (400 mg) en dose unique à répéter chaque année permet d'abaisser les charges microfilariennes du patient mais n'a pas ou peu d'effets sur les signes cliniques de l'infection.
- Dans les pays où l'onchocercose n'est pas endémique, l'association diéthylcarbamazine (DEC) (6 mg/kg) et albendazole (400 mg) en dose unique à répéter chaque année permet également d'abaisser les charges microfilariennes du patient mais n'a pas ou peu d'effets sur les signes cliniques de l'infection.

L'objectif principal de ces traitements est de maintenir la microfilarémie des sujets infectés à un niveau très faible, ce qui permet d'interrompre la transmission.

La sélection des populations-cibles de ces campagnes d'administration massive pour la filariose lymphatique reposait sur l'identification aléatoire de 2 villages par district sanitaire et l'utilisation d'indicateurs épidémiologiques permettant de définir le niveau d'endémicité d'une région pour la filariose lymphatique. Historiquement, la prévalence des hydrocèles dans la population masculine était utilisée pour réaliser des cartographies rapides des niveaux d'endémicité. Cette méthode a été abandonnée et remplacée par un indicateur parasitologique et un indicateur immunologique. Le premier est basé sur la mesure de la prévalence de la microfilarémie dans une communauté via la détection de microfilaires en examen direct, par goutte épaisse calibrée. L'indicateur immunologique permet de mesurer la fréquence des

porteurs de vers adultes dans une population grâce à l'utilisation des tests détecteurs d'antigènes filariens circulants. Cet indicateur est utilisé pour définir les zones éligibles aux programmes de lutte. On considère une zone comme éligible quand la prévalence des antigènes filariens circulants ou de la microfilarémie est supérieure ou égale à 1%.

Le principal problème qui s'est posé au cours du GPELF concerne les zones endémiques pour la loase. En effet, comme expliqué précédemment, cette endémicité rend impossible l'utilisation d'ivermectine. En 2012, l'OMS a proposé de distribuer dans ces zones de l'albendazole seul, tous les 6 mois. Cette stratégie a été validée par plusieurs études évaluant l'impact communautaire de cette distribution – elle permettrait d'abaisser la prévalence de l'antigénémie et la microfilarémie dans les communautés traitées (Pion et al. 2015, 2017, 2020) .

En 2017, il était estimé que sur les 51 pays nécessitant un traitement, 46 avaient des programmes d'administration en cours avec environ 465 millions de personnes traitées depuis l'instauration du GPELF. De plus, la prise en charge individuelle des incapacités liées à la maladie étaient prises en charge dans 38 de ces pays.

Le choix d'arrêter la distribution des médicaments suit un algorithme de décision. Après 5 traitements de masse avec un couverture thérapeutique supérieure ou égale à 65%, une mesure de la prévalence ou de l'antigénémie était effectué dans la population générale des villages choisis lors de la sélection des populations-cibles. Dans ces villages, si la prévalence des microfilaires est inférieure à 1% ou si la prévalence de l'antigénémie est inférieure à 2%, une enquête d'évaluation de la transmission (*Transmission Assessment Survey* ; TAS) est réalisée. Cette enquête porte sur la mesure de la prévalence de l'antigénémie dans la population des enfants âgés de 6 à 7 ans. Si cette mesure est jugée adéquate, les distributions de traitement sont arrêtées. En 2017, il a été mesuré que 500 millions d'individus vivant dans 44 pays différents ont pu bénéficier de l'arrêt des campagnes de distribution.

### 1.3.2 Situation des programmes en 2021

Les programmes de lutte contre l'onchocercose et la filariose lymphatique sont des succès indéniables. L'APOC a permis de réduire drastiquement les DALYs de l'onchocercose (Coffeng, Stolk, Zouré, et al. 2014) (figure 8).

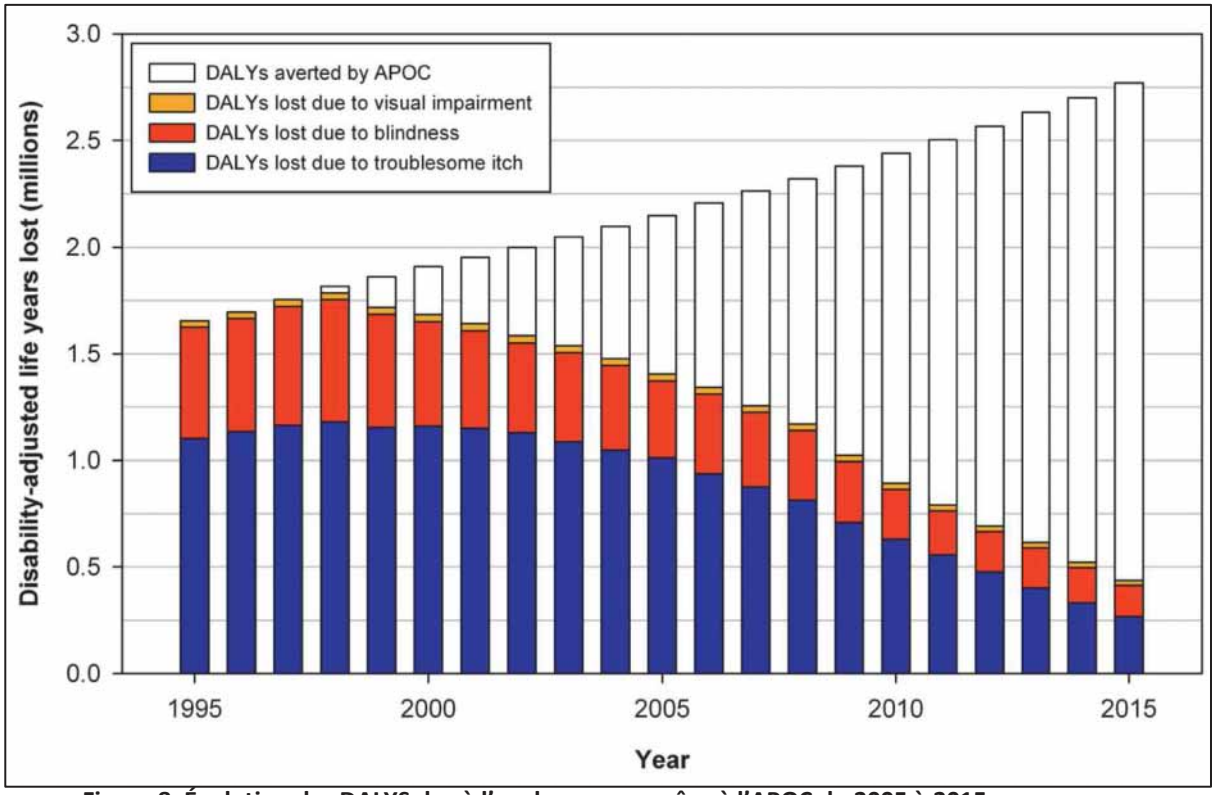



**Figure 8. Évolution des DALYS dus à l'onchocercose grâce à l'APOC de 2005 à 2015**

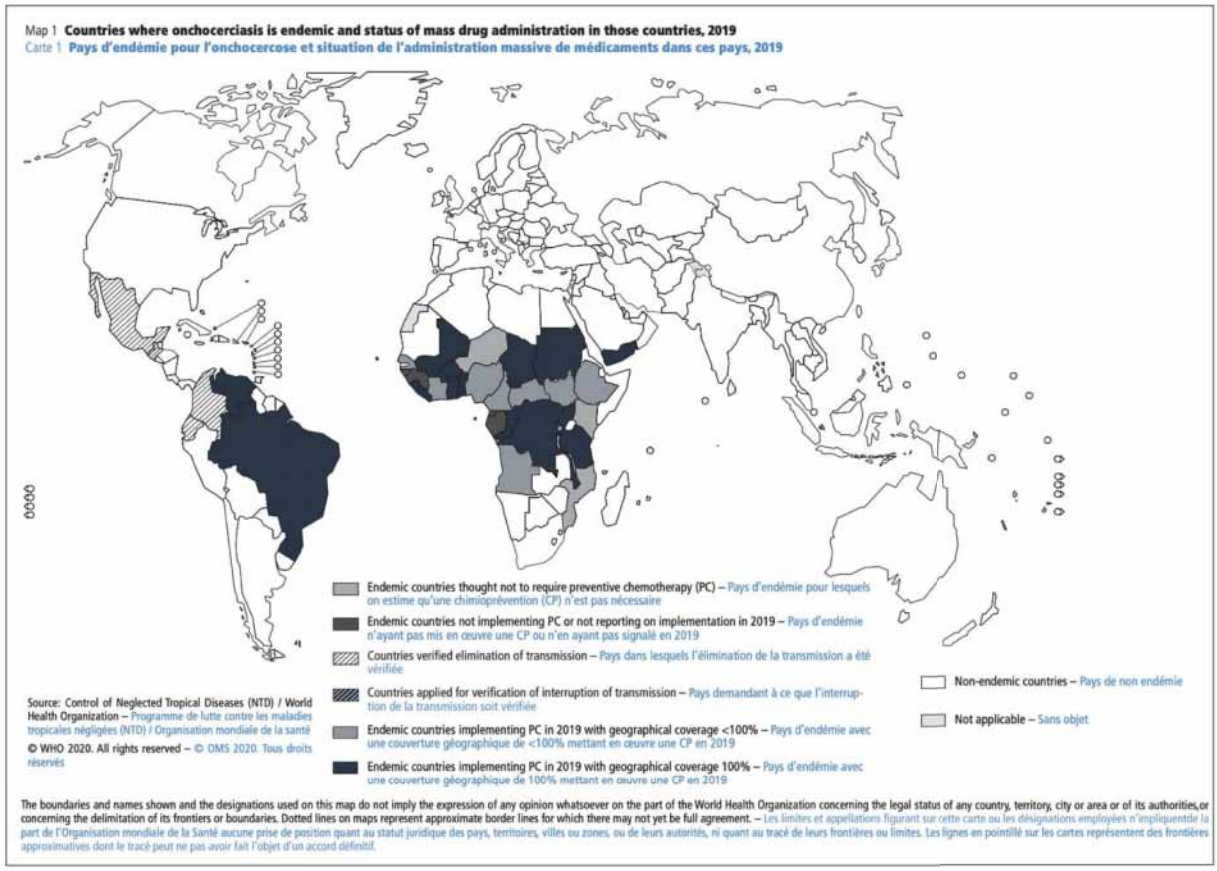



**Figure 9. Situation de l'administration massive de médicaments dans le monde pour l'année 2019**

En novembre 2020, l'OMS publia un rapport de situation faisant l'état des lieux des programmes mondiaux de lutte contre l'onchocercose (Organisation Mondiale de la Santé 2020a). En 2019, plus de 150 millions de personnes ont reçu un traitement contre l'onchocercose en Afrique sur un objectif de 217,2 millions de personnes à traiter pour atteindre une couverture géographique de 100%. Parmi les 26 pays africains concernés par les programmes, 14 ont atteint une couverture géographique de 100% (figure 9).

A l'instar de l'onchocercose, l'OMS a publié un rapport de situation sur la filariose lymphatique pour l'année 2019 (Organisation Mondiale de la Santé 2020b). Trois pays ont déclaré avoir éliminé la filariose lymphatique comme problème de santé publique au cours de l'année 2019 : le Kiribati, le Malawi et le Yémen. Sur les 72 pays endémiques pour la filariose lymphatique, 50 nécessitent encore des campagnes d'administration massive de médicament. En Afrique, sur les 34 pays concernés, 3 n'ont pas encore commencé les campagnes d'administration, 4 ont commencé les campagnes mais ne les ont pas étendues à toutes les régions endémiques, 25 ont des campagnes d'administration dans toutes les régions endémiques et enfin, 2 sont en phase de surveillance après avoir valider l'élimination de la filariose lymphatique comme problème de santé publique. Depuis 2000, plus de 920 millions de personnes ont bénéficié de traitements dans le cadre des campagnes d'administration.

### 1.3.3 Stratégies alternatives de traitements développées et évaluées

#### *1.3.3.1 Stratégie IDA : stratégie pour les zones endémiques pour la filariose lymphatique uniquement*

Afin d'accélérer l'élimination de la filariose lymphatique et dans un souci d'intégration des programmes de lutte, en 2017, l'OMS a recommandé l'utilisation de 3 molécules (IDA pour Ivermectine, Diéthylcarbamazine et Albendazole) dans les zones où les campagnes reposaient initialement sur la bithérapie DEC et albendazole (DA). Cette association a montré son efficacité lors de deux essais cliniques s'intéressant la négativation de la microfilarémie à *W. bancrofti* chez des populations fortement infectées. En effet, 96% des personnes participant à l'essai et prenant le traitement IDA n'avait plus de microfilaires après 3 ans de traitement (Thomsen et al. 2016;

King et al. 2018) et la plupart des participants restaient amicrofilarémiques 5 après une prise unique d'IDA (King et al. 2020).

Les effets secondaires imputables à la stratégie IDA ont également été comparé aux autres stratégies par le projet DOLF (*Death to Onchocerciasis and Lymphatic Filariasis*), un consortium de chercheurs financé par l'association Bill & Melinda Gates. Une étude communautaire sur près de 26.000 personnes a permis de conclure que les types et la gravité des effets secondaires étaient identiques après une stratégie IDA ou une stratégie DA (Weil et al. 2019).

L'utilisation de la stratégie IDA permettrait donc d'accélérer l'élimination de la filariose lymphatique mais du fait de la présence notamment de la DEC, seules les zones endémiques uniquement pour la filariose lymphatique peuvent être concernées par cette stratégie.

#### *1.3.3.2 Test & (not) Treat : stratégie pour les zones endémiques pour l'onchocercose et coendémique pour la loase*

Parmi les différentes stratégies alternatives développées pour permettre de réaliser l'objectif de l'APOC d'éliminer l'onchocercose d'ici 2025, la stratégie *Test & Treat* (TNT) est envisagé (Boussinesq et al. 2018).

Cette stratégie aurait un intérêt dans les zones hypoendémiques à l'onchocercose. La première stratégie TNT à avoir pu être évaluée repose sur l'identification des personnes à risque de développer un effet secondaire grave post-ivermectine. Pour cela, la stratégie TNnT repose sur la réalisation sur le terrain du LoaScope, qui permet d'afficher en quelques minutes si la personne présente plus de 20 000 mf/mL à *L. loa* ou non ; si c'est le cas, la personne est exclue du traitement par ivermectine, et sinon, la personne est éligible à recevoir un traitement à base d'ivermectine (Kamgno et al. 2017). Comme alternative pour les exclus, un traitement par doxycycline pendant 5 semaines peut être envisagé, mais avec toutes les difficultés liées à l'observance que cela entraine.

Une étude récente montre que toutes les personnes traitées par l'ivermectine durant cette stratégie sont toujours sous le seuil des 20 000 mf/mL plus de 18 mois après (Pion et al. 2019) ; permettant alors d'imaginer appliquer secondairement un traitement de masse sans LoaScope lors des campagnes suivantes (sauf pour les exclus), simplifiant alors la logistique et diminuant les coûts.

#### *1.3.3.3 Optimisation des schémas d'administration d'albendazole : zones coendémiques pour la filariose lymphatique et la loase*

Le projet DOLF (*Death to Onchocerciasis and Lymphatic Filariasis*), porté par le Professeur Gary Weil de l'Université de Washington à Saint Louis aux USA, en plus de ses missions de développement de nouveaux stratégies médicamenteuses pour l'administration massive de médicaments comme la stratégie IDA, est également impliqué dans l'optimisation des stratégies médicamenteuses déjà existantes.

DOLF a permis de mettre en place des essais communautaires évaluant l'intérêt des campagnes d'administration semi-annuel plutôt qu'annuel de DEC et d'albendazole pour lutter contre la filariose lymphatique. Les résultats indiquent que l'administration annuelle était suffisante pour réduire la prévalence de la microfilarémie à moins de 1% dans les zones où l'endémicité était faible à modérée, mais que l'administration semi-annuelle semblait intéressante pour réduire plus rapidement la prévalence de la microfilarémie dans les zones hyperendémiques pour la filariose lymphatique (Supali et al. 2019).

Concernant les zones d'endémie à la loase, DOLF a également permis d'évaluer la stratégie alternative de traitement de masse proposée par l'OMS en 2012 : l'administration semi-annuel d'albendazole seul. Les résultats obtenus sur 2 essais communautaires en République du Congo et en République Démocratique du Congo montrent que cette stratégie est sûre et efficace pour réduire la prévalence de l'antigénémie et de la microfilarémie à filariose lymphatique, mais également pour réduire les prévalences communautaires des infections (Pion et al. 2015, 2017, 2020).

# 2. Problématiques et objectifs

## 2.1 Les zones endémiques à la loase et les stratégies déployées

Pour imaginer une éradication de l'onchocercose et de la filariose lymphatique d'ici 2030, il faut mettre en place des stratégies alternatives pour (i) lutter contre ces filarioses dans les régions où elles sont hypoendémiques et coendémiques pour la loase et (ii) accélérer la lutte contre ces filarioses dans les régions où elles sont méso- ou hyperendémiques et coendémiques pour la loase.

### 2.1.1 Outils thérapeutiques pour l'optimisation des programmes de lutte

#### *2.1.1.1 Le patch de diéthylcarbamazine*

Concernant les stratégies permettant de répondre à l'objectif : lutter contre l'onchocercose dans les régions où elle est hypoendémique, nous avions présenté la première stratégie TNT qui repose sur l'identification des personnes à risque de développer un effet secondaire grave post-ivermectine. La seconde stratégie TNT intéressante serait de dépister en premier les individus réellement infectés par *O. volvulus* (Boussinesq et al. 2018). Dans les zones hypoendémiques, la réalisation des BCE ne paraît pas envisageable à large échelle. Ce geste, relativement invasif, s'il est réalisé dans une population où la prévalence de l'onchocercose est très basse, pose de sérieux problèmes éthiques et d'acceptabilité de la population. L'alternative serait donc d'utiliser un test moins invasif comme les ELISA et les tests immunologiques. Le problème de ces tests est qu'ils ne permettent pas de faire la distinction entre les personnes ayant une infection active ou passée. Ils sont donc inutilisables dans ce contexte de TNT. Une autre possibilité repose sur la réaction de Mazzotti au niveau local grâce à l'utilisation d'un patch imprégné en DEC. Si l'individu est infecté par *O. volvulus*, la DEC va tuer localement les microfilaires d'*O. volvulus* et induire de petites papules détectables environ 24 heures après la pose du patch. La première version de ce DEC-patch a été conçue par le Programme de Lutte contre l'onchocercose en Afrique de l'Ouest (OCP). Il s'agit d'une préparation magistrale composée de DEC dissoute dans de la crème hydratante (Nivea®). Cette préparation était

appliquée sur la peau puis entourée par un film plastique (Toè et al. 2000). Ce DEC-patch avait de bonnes performances mais son manque de standardisation et sa difficulté à mettre en place l'a rendu obsolète. Plus récemment, un DEC-patch standardisé a été fabriqué et commercialisé par Lohmann Therapie System®. Ce LTS-2 patch s'est avéré facile à utiliser et hautement spécifique de l'onchocercose dans une étude menée en Afrique de l'Ouest sur plus de 2000 personnes négatives au diagnostic direct des BCE (Diawara et al. 2009). Son innocuité a été évaluée dans une étude menée au Ghana sur des adultes présentant une charge microfilarienne onchocerquienne faible (<40 mf/snip) (Awadzi et al. 2015). Cependant, avant d'être utilisé, il est primordial de vérifier son innocuité dans une population présentant des charges microfilariennes plus importantes. Il est également nécessaire d'évaluer sa spécificité, et notamment dans les zones où la loase est coendémique puisqu'il a été démontré que le patch de l'OCP se positivait en cas de forte charge microfilarienne en *L. loa* (Ozoh et al. 2007), mais ce patch contenait environ 40 mg de DEC alors que le patch LT2 n'en contient que 5,4 mg. En raison de la pandémie due au COVID-19, ces travaux n'ont pas pu être intégrés dans ces travaux de thèse et feront l'objet d'un travail ultérieur.

#### *2.1.1.2 Administration d'albendazole seul semi-annuel*

Parmi les stratégies permettant de répondre à l'objectif de lutter contre la filariose lymphatique dans les zones où la loase est coendémique, nous avons présenté la stratégie visant à traiter les populations par albendazole seul tous les 6 mois. Cette proposition de stratégie, émanant de l'OMS, reposait sur deux notions : (i) l'albendazole n'a jamais été rapporté comme pouvant induire des effets secondaires graves chez les sujets ayant une microfilarémie élevée à *L. loa* (Kamgno et al. 2016; Tabi et al. 2004; Tsague-Dongmo et al. 2002) et (ii) les résultats d'essais cliniques ayant comparé l'effet de l'albendazole seul et celui de la combinaison ivermectine & albendazole sur la microfilarémie à *W. bancrofti* suggéraient que le traitement par albendazole seul pouvait également réduire la microfilarémie, bien que plus lentement que la combinaison ivermectine et albendazole (Ismail et al. 2001; Makunde et al. 2003; Panicker et al. 1991; Dembele et al. 2010; Kazura 2010; Cartel et al. 1991, 1992; Fox et al. 2005; Gayen et al. 2013; Dunyo et al. 2000; Addiss et al. 1997). En parallèle à cette proposition, deux essais communautaires en République du Congo et en République Démocratique du Congo ont, pour la première fois, évalué l'impact, en vie réelle, de l'administration massive semi-annuelle

d''albendazole seul au niveau communautaire. Ces essais ont démontré que cette stratégie pouvait être un franc succès pour prendre en charge les zones de coendémie à la filariose lymphatique et à la loase puisqu'elle a permis de réduire la prévalence de l'antigénémie et la prévalence de la microfilarémie de manière significative, après 3 ans de traitement dans une population où la prévalence initiale était modérée (Pion et al. 2017) et après 4 ans dans une population où la prévalence initiale était élevée (Pion et al. 2020). De plus, l'administration semi-annuelle d'albendazole à toute la population a également permis de réduire les niveaux d'infection par géohelminthes. Néanmoins, sans remettre en doute l'intérêt avéré de cette stratégie au niveau communautaire, certaines hypothèses sont encore à vérifier. En effet, toutes les études sur le sujet ont raisonné d'un point de vue communautaire et aucune étude ne s'est intéressé à l'impact de cette stratégie au niveau individuel. De plus, aucune étude n'a utilisé un groupe contrôle pour évaluer cette stratégie.

Il semble exister un phénomène de lassitude au sein des communautés qui provoquent une diminution de la compliance thérapeutique au cours du temps, ce qui peut ralentir l'élimination des infections. Enfin, une hétérogénéité considérable a été observée dans la disparition des infections par la filariose lymphatique et par les géohelminthes au niveau individuel ; certains individus ont éliminé leurs infections après une seule prise d'albendazole, tandis que d'autres sont restés infectés après plusieurs campagnes d'administration. Les travaux de thèse présentés au Chapitre 3 portent sur ce phénomène, et notamment sur l'évaluation de l'effet de la compliance thérapeutique individuelle aux programmes d'administration de masse sur le temps nécessaire à la disparition des indicateurs de l'infection à *W. bancrofti*.

#### *2.1.1.3 La moxidectine*

La moxidectine est une molécule à la structure similaire de celle de l'ivermectine. Elles diffèrent notamment par leur temps de demi-vie et leur mode d'action principal (Prichard et al. 2012) – la moxidectine possède une demi-vie bien plus longue (de l'ordre de 20 jours) que l'ivermectine (de l'ordre de 20 heures). La moxidectine aurait donc un intérêt majeur pour la réalisation des objectifs d'élimination de l'onchocercose, par sa durée d'action plus longue, elle permettrait une réduction de la transmission du parasite entre les traitements annuels communautaires. Elle serait donc une alternative aux TIDC dans les zones où un besoin d'accélération de l'élimination est nécessaire, où des barrières opérationnelles empêchent

l'administration massive régulière de médicaments et possiblement dans les zones où la loase est endémique grâce à une stratégie de TNT.

Des études de phase I sur volontaires sains et de phase II et III sur des patients infectés par l'onchocercose ont déjà permis d'évaluer la pharmacocinétique et l'efficacité supérieure de la moxidectine chez l'homme en comparaison à l'ivermectine (Opoku et al. 2018). Le dernier essai clinique réalisé était un essai contrôlé, randomisé en double aveugle de phase III, lancé en 2009, il a permis de déterminer la supériorité de la moxidectine par comparaison à l'ivermectine ; la charge en microfilaires dermiques est significativement inférieure 12 mois après une prise unique de moxidectine. La diminution de la charge microfilarienne d'*O.volvulus* est bien plus rapide et dure plus longtemps après une dose de 4 ou 8 mg de moxidectine en comparaison à la dose conseillée d'ivermectine. La moxidectine semble donc être un choix pertinent pour remplacer l'ivermectine et accélérer l'élimination de l'onchocercose. Des études médico-économiques ont évalué l'intérêt financier d'instaurer un traitement annuel par la moxidectine en comparaison à un traitement annuel ou biannuel à l'ivermectine (Turner et al. 2015). Il en résulte que, la moxidectine a une efficacité au moins similaire à un traitement biannuel par ivermectine ; mais que, contrairement à la stratégie biannuelle d'administration d'ivermectine, le coût engendré par une campagne annuelle d'administration de moxidectine n'entrainerait pas de surcoût par rapport à un TIDC annuel. De plus, le moment où le traitement par la moxidectine est administré n'est pas influencé par schémas saisonniers de transmission – ce qui engendrerait moins de contraintes logistiques. Un des derniers points à évaluer, et non des moindres, est d'estimer la tolérance de la moxidectine chez des individus coinfectés par *L. loa* pour déterminer si un traitement de masse par moxidectine est envisageable en zone endémique pour la loase. En raison de la pandémie due au COVID-19, ces travaux n'ont pas pu être intégrés dans ces travaux de thèse et feront l'objet d'un travail ultérieur.

### 2.1.2 Le lévamisole

Le lévamisole est une molécule découverte en 1966 par le laboratoire Janssen®, dérivé de l'imidazothiazole. Il fait partie de la liste des médicaments essentiels de l'OMS depuis avril 2013. Il a également été utilisé pour ses propriétés immunomodulatrices en tant qu'adjuvant de chimiothérapie et dans certains syndromes néphrotiques de l'enfant. Enfin, le lévamisole a été

mésusé comme produit de coupe de la cocaïne. En France et aux États-Unis, le lévamisole a perdu son AMM à cause des potentiels effets secondaires graves qu'il pourrait provoquer mais d'autres pays l'utilisent encore quotidiennement pour son indication antiparasitaire, généralement en dose unique, et ne semblent pas signaler d'effets indésirables graves. Une partie de cette thèse s'intéressa aux effets secondaires rapportés après la prise de lévamisole et à l'analyse de leur fréquence de notification selon le type d'utilisation du lévamisole (paragraphe 5.2).

En Afrique Centrale, le lévamisole est toujours utilisé sous le nom Decaris® pour son action sur les géohelminthes. Cependant, son action sur les filaires n'a été que très peu étudié. Il est décrit comme relativement efficace sur la filariose lymphatique (Miller 1980), peu efficace sur l'onchocercose (Awadzi et al. 2004) et n'a jamais été testé sur la loase.

L'intérêt potentiel d'un traitement par lévamisole serait d'utiliser sa faible action microfilaricide pour permettre de traiter la population infectée par *L. loa* en vue de diminuer progressivement la charge microfilarienne en *L. loa* en dessous du seuil d'apparition d'effets indésirables graves, et d'ensuite traiter l'ensemble de la population par l'ivermectine, sans risquer l'apparition d'effets secondaires graves.

Le chapitre 5 de cette thèse s'intéressera à cette molécule grâce à l'analyse d'un essai clinique évaluant la tolérance et l'efficacité du lévamisole sur la charge microfilarienne de *L. loa* et à l'analyse de la base mondiale de l'OMS recensant les effets secondaires rapportés suite à la prise de médicaments.

## 2.2 L'ivermectine, effets prophylactiques et risques associés

### 2.2.1 Effet prophylactique de l'ivermectine

Découverte dans les années 1980 par S. Omura, W.C. Campbell et T. Youyou, l'ivermectine est probablement la pierre angulaire de la lutte contre les filarioses. Son mécanisme d'action est partiellement connu, on sait qu'elle agit par action sur les canaux chlore dépendants du glutamate présents sur les muscles et le pharynx des vers. Ce qui engendre deux effets principaux : un effet microfilaricide, c'est-à-dire un effet direct sur les microfilaires présentes dans la peau, un effet embryostatique, c'est-à-dire qu'elle empêche la libération de nouvelles microfilaires par les vers femelles adultes pendant plusieurs mois. Cependant, l'effet macrofilaricide, c'est-à-dire l'effet sur la viabilité des vers adultes d'*O. volvulus* et *W. bancrofti* est considéré comme modéré, il en résulte que les traitements doivent être répétés afin de maintenir les densités microfilaires cutanées à des niveaux faibles non associés à des manifestations cliniques. Cependant, peu d'informations sont disponibles sur l'effet prophylactique de l'ivermectine (Lämmler 1977), c'est-à-dire l'effet sur les larves immatures de vers adultes et les larves au troisième et quatrième stade. Si un effet prophylactique existe, même faible, (i) les TIDC annuels pourraient potentiellement être remplacés par des campagnes d'administration plus rapprochés afin de jouer sur cet effet prophylactique de l'ivermectine et donc sur l'apparition des vers adultes, et (ii) une recommandation médicale pourrait être faite dans cette indication, notamment pour les expatriés se rendant en zone endémique. Une partie du chapitre 4 de cette thèse s'intéressera à cet effet prophylactique.

### 2.2.2 Les effets secondaires de l'ivermectine

L'ivermectine est un médicament utilisé dans le monde entier pour diverses indications : onchocercose, filariose lymphatique, pédiculose, *larva migrans* de l'ankylostomose, strongyloïdose, gale sarcoptique humaine, acarodermatite et rosacée. Au début des années 1990, l'ivermectine a été incriminée dans la survenue d'encéphalopathies graves chez certains

patients d'Afrique Centrale présentant une forte charge parasitaire de *L. loa* (Ducorps et al. 1995). Peu d'études ont été menées pour étudier la fréquence de ces effets indésirables liés à la loase, où les mécanismes par lesquels ils se produisent. Dans le chapitre 4 de ces travaux de thèse, nous nous sommes intéressés à réaliser une synthèse de tous les effets indésirables graves signalés comme imputables à l'ivermectine dans la base de pharmacovigilance de l'OMS, afin de comparer les cas selon qu'ils sont ou non originaires de zones où la loase est endémique.

## 2.3 Périodicité et variabilité de la loase

### 2.3.1 Périodicité

La présence des microfilaires de *L. loa* dans le sang suit une périodicité diurne chez l'homme tandis qu'elle suit, vraisemblablement, une périodicité nocturne chez les singes (Hawking 1955). La présence d'une telle périodicité impose, dans les études longitudinales portant sur la loase, de réaliser les prélèvements à la même heure chez les patients engendrant un besoin en techniciens supplémentaire et donc des coûts. Dès lors si, pour des raisons logistiques, les prélèvements n'ont pas pu être réalisés à des heures similaires, il n'est pas possible d'attribuer la différence de microfilarémie à l'intervention ou à la périodicité du parasite. De plus, il est possible que la prise de décision quant à la dispensation d'un traitement microfilaricide chez certains patients puissent être impactée par cette périodicité. Peu d'étude se sont intéressés à la description (Hawking et al. 1964, 1967; Hawking 1955) ou à la modélisation mathématique (Kamgno et al. 2009) de cette périodicité.

Une partie du chapitre 6 de ces travaux cherchera à synthétiser les différentes informations sur le sujet et à la présentation des résultats d'une étude réalisée en République du Congo et s'intéressant à l'évaluation de cette périodicité au cours d'une journée et à sa modélisation chez 13 patients microfilarémiques.

### 2.3.2 Variabilité de la microfilarémie à *Loa loa* dans le temps

Par définition, la microfilarémie a *L. loa* est définie comme stable dans le temps lorsqu'aucun traitement n'est donné aux patients (Carme 1983; Pion et al. 2019). Cependant, peu d'études se sont intéressées au phénomène de variabilité dans le temps de cette microfilarémie et aux facteurs pouvant l'influencer. La plupart de ces études sont anciennes et n'avaient pas encore accès aux logiciels de modélisation mathématique.

De manière similaire à la périodicité journalière, la variabilité temporelle de la microfilarémie à *L. loa* entraine des difficultés logistiques à la réalisation d'études longitudinales, notamment pour l'évaluation d'interventions pouvant diminuer la microfilarémie au cours du temps.

Lors de l'essai clinique portant sur l'évaluation du lévamisole contre placebo sur la loase en République du Congo, nous avons été confrontés à deux problématiques portant sur la variabilité de la microfilarémie au cours du temps et ayant compliqué l'inclusion et l'évaluation du lévamisole par comparaison au placebo.

Une partie du chapitre 6 de ces travaux s'intéressera à la synthèse des études antérieures sur le sujet ainsi qu'à la présentation d'une nouvelle étude s'intéressant à la variabilité à court (jours et semaines) et long terme (année) de la microfilarémie à *L. loa*.

### 2.3.3 Variabilité de lecture de microfilarémie dans les infections à *Loa loa*

Le diagnostic de la loase repose sur l'examen direct de microfilaires dans le sang périphérique en microscopie optique à partir d'une goutte épaisse calibrée de sang. En pratique, un prélèvement est réalisé au bout du doigt du patient, une quantité standardisée est collectée grâce à un capillaire à hématocrite puis est déposée et étalée sur une lame porte-objet. La lame est mise à sécher à température ambiante puis colorée au Giemsa. Le technicien lit ensuite la lame entière en comptant les microfilaires puis multiple la quantité retrouvée pour atteindre une quantité en nombre de microfilaires par millilitre de sang. Ce travail est laborieux, notamment lorsque la charge parasitaire est élevée puisqu'on estime à environ 20 minutes le temps de lecture d'une seule lame pour un patient avec un nombre de microfilaires modéré à important, ce qui peut engendrer des variations de résultats en fonction du nombre de lames consécutivement lues par le technicien. En effet, il est estimé à environ 20 le nombre de lames maximales lues par un technicien par jour sans risque de variabilité importante intra-individuelle. Enfin, lors des diagnostics par double lecture une hétérogénéité des résultats se fait ressentir en fonction du lecteur, nécessitant parfois une troisième lecture des lames.

Lors de l'essai clinique du lévamisole, pour chaque prélèvement de goutte épaisse, nous avons réalisé 2 lames qui ont chacune été lues par 2 microscopistes qualifiés en aveugle. Malgré

leur expérience dans la lecture de lames issues de gouttes épaisses calibrées pour quantifier les microfilarémies à *L. loa*, des différences importantes ont pu être constaté sur certaines lames. Cette hétérogénéité de lecture n'a jamais été quantifiée auparavant. Le chapitre 6 de ces travaux cherchera à évaluer pour la première fois cette variabilité intra- et interindividuelle.

A la suite du temps dédié à l'essai clinique du lévamisole, nous avons réalisé une série de protocoles de recherche afin d'essayer de répondre à certaines de ces problématiques.
Une première étude ancillaire a été d'évaluer la périodicité de la microfilarémie à *L. loa* au cours d'une journée afin de la comparer à celles décrites dans la littérature scientifique et à étudier si des facteurs extérieurs ont une influence sur celle-ci.
L'objectif d'une deuxième et troisième étude ancillaire a été d'étudier la variabilité à court terme et à long terme de la microfilarémie à *L. loa* et d'évaluer si des facteurs extérieurs étaient associés à cette dernière chez certains patients.
Enfin, une dernière étude ancillaire s'est intéressée directement à la quantification de la variabilité des lectures microscopiques selon certains des critères usuellement utilisés par les organismes d'accréditation type COFRAC.

**La figure 10 résume le contexte de recherche, les stratégies alternatives, les problématiques et les objectifs de recherche qui seront présentés au cours de ce manuscrit.**

Ces travaux chercheront tout d'abord à apporter des éléments de réponse concernant l'évaluation de l'efficacité de l'administration semi-annuelle d'albendazole contre la filariose lymphatique, l'évaluation des effets prophylactiques et des effets secondaires graves de l'ivermectine, l'évaluation de la tolérance et de l'efficacité du lévamisole dans la prise en charge de la microfilarémie à *Loa loa*, et termineront par des études portant sur la périodicité et la variabilité à court terme, à long terme et dans le diagnostic quantitatif de la microfilarémie à *Loa loa*.

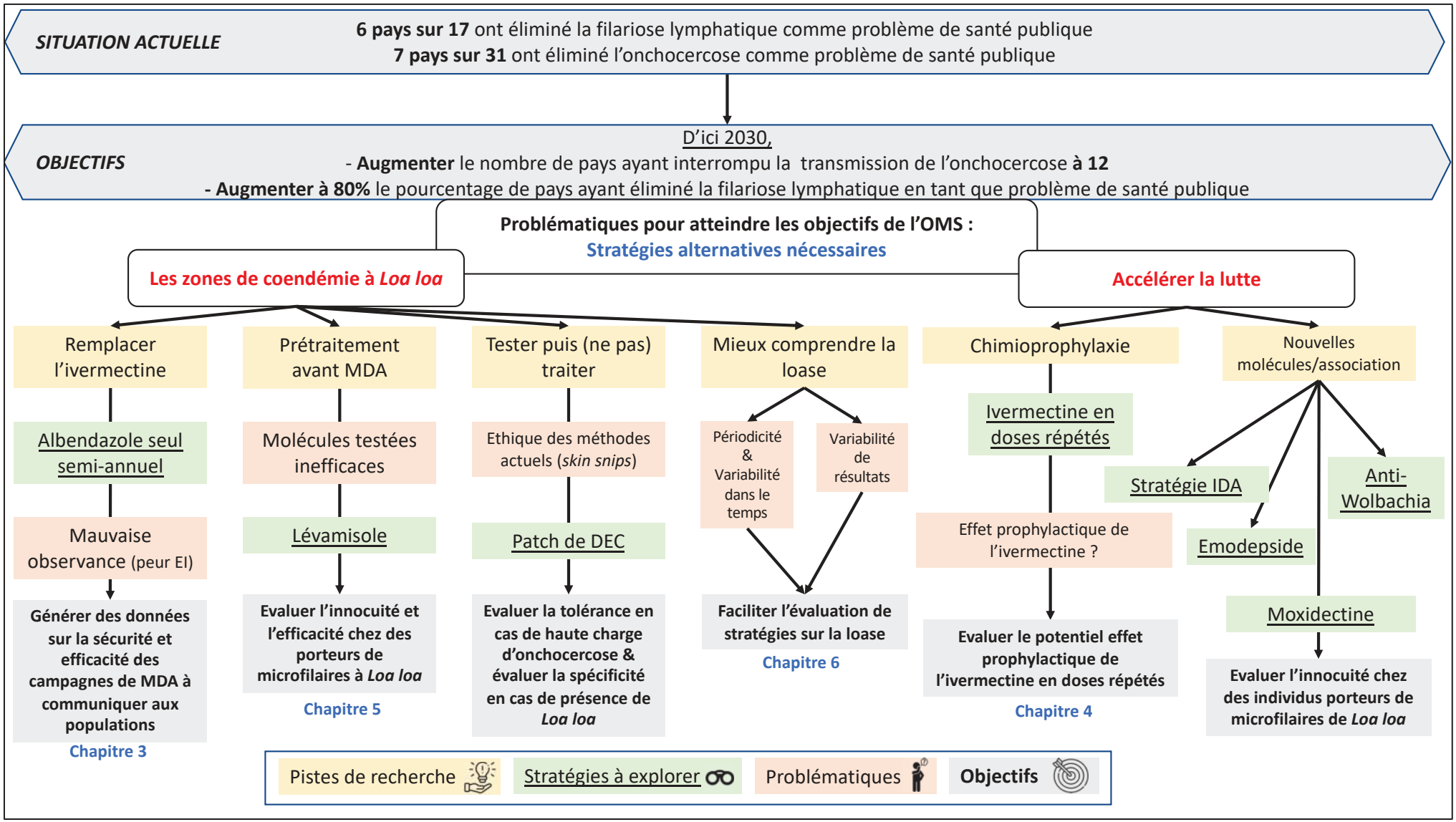



**Figure 10. Résumé des stratégies alternatives qui seront développées dans ce manuscrit**

EI, effet secondaire ; MDA, *mass drug administration* ; DEC, diéthylcarbamazine ; IDA, ivermectine-diéthylcarbamazine-albendazole

# 3. Éléments de réponse sur l'intérêt des programmes d'administration semi-annuelle d'albendazole seule sur la filariose lymphatique

## 3.1 Mécanisme et indications de l'albendazole

Breveté en 1975, l'albendazole ou le 5-propylthio-1H-benzimidazol-2-yl-carbamic acid-méthyl-ester (Figure 11) a été découvert par Robert J. Gyurik et Vassilios J. Theodorides (Gyurkik and Theodorides 1975). Il s'agit d'une molécule chimique de la classe des benzimidazolés. En 1977, l'albendazole est indiqué comme antihelminthique vétérinaire. Il a fallu attendre 1982 pour que son utilisation chez l'humain soit acceptée (Turner and Horton 1987) et 2013 pour que l'OMS l'introduise dans la liste des médicaments essentiels (World Health Organization 2019).

De nos jours, l'albendazole est utilisé dans de multiples infections parasitaires. Le Résumé des Caractéristiques du Produit (RCP) français indique : l'oxyurose, l'ascaridiose, l'ankylostomose, la trichocéphalose, l'anguillulose, le ténia, les giardioses et la trichinellose (ANSM 2010).

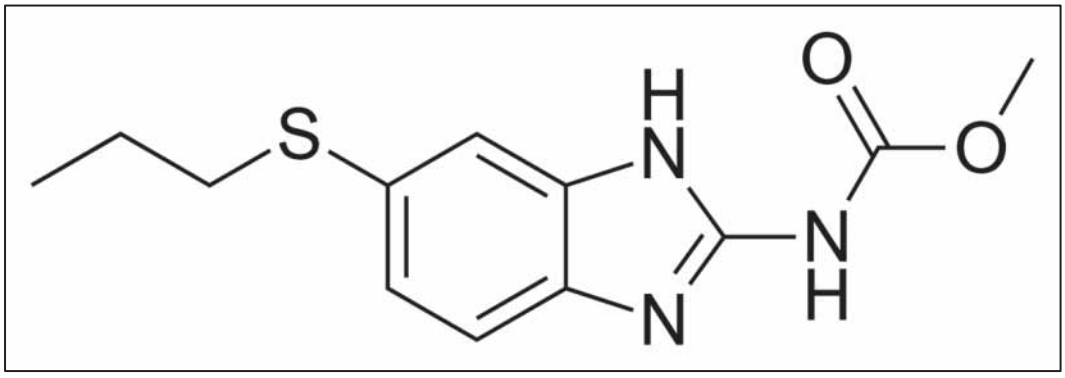



**Figure 11. Structure chimique de l'albendazole**

L'action antiparasitaire de l'albendazole est due à divers mécanismes dont les principaux sont : (i) l' altération des cellules intestinales des vers via sa liaison au site sensible à la colchicine de la β-tubuline, inhibant ainsi sa polymérisation ou son assemblage en microtubules, (ii) l'inhibition de l'absorption du glucose chez certains parasites, et de fait, l'épuisement de leurs réserves de glycogène et (iii) l'inhibition de la division cellulaire des vers qui engendre un blocage de la production et du développement des œufs (DRUGS 2020). Ces multiples mécanismes d'action sur l'organisme des parasites fait de l'albendazole un médicament efficace dans de nombreuses autres indications parasitaires que celles proposées par le RCP français, comme la filariose lymphatique.

## 3.2 Utilisation de l'albendazole dans la lutte contre la filariose lymphatique

### 3.2.1 Avis de l'OMS

L'administration massive d'albendazole a toujours fait partie intégrante des programmes de lutte contre la filariose lymphatique. En 2005, une revue Cochrane des essais portant sur l'efficacité d'une dose unique d'albendazole sur la filariose lymphatique a conclu qu'une dose unique d'albendazole seule n'était pas plus efficace qu'un placebo pour éliminer la microfilarémie à *W. bancrofti* (Addiss et al. 2005).

En 2012, l'OMS proposa l'utilisation d'albendazole seul deux fois par an dans les zones où la loase est endémique puisque la balance bénéfice-risque de l'administration massive d'ivermectine ou de la DEC empêche leurs administrations.

Le rationnel de la proposition de l'OMS était principalement basé sur la capacité de l'albendazole de diminuer la microfilarémie de manière plus progressive que l'association ivermectine et albendazole, permettant ainsi une administration massive dans une population où des individus sont potentiellement fortement chargés en loase (Dembele et al. 2010; Kazura 2010). De plus, aucun effet secondaire grave n'a été rapporté lors des différents essais d'administration d'albendazole sur des individus possédant de hautes microfilarémie à *Loa loa* (Tsague-Dongmo et al. 2002; Tabi et al. 2004; Kamgno et al. 2016).

### 3.2.2 Effet au niveau communautaire

Parallèlement à cette proposition de l'OMS, le projet DOLF et l'équipe de recherche Maladies Tropicales négligées de l'IRD avaient lancé deux essais communautaires pour évaluer l'impact de l'administration semi-annuelle d'albendazole dans les zones coendémiques à la loase et à la filariose lymphatique. Le premier a pris place dans un village de la République du Congo appelé Séké Pembe de 2012 à 2015. Le deuxième a pris place dans deux villages de la République Démocratique du Congo : Misay et Mbunkimi de 2014 à 2018.

Avant l'intervention, les niveaux d'infection à Séké Pembe étaient modérés pour la filariose lymphatique (prévalence de l'antigénémie : 17.3%), faibles pour l'ankylostome (6.5%) et importants pour la trichocéphalose (78.6%) et l'ascaridiose (56.5%). La première évaluation fut effectuée douze mois après la mise en place de l'essai, soit après 2 campagnes d'administration. Il n'a pas été mis en évidence de diminution significative de l'antigénémie ou de la microfilarémie à *Wuchereria bancrofti* dans la communauté (de 17,3 % à 16,6 % et de 5,3 % à 4,2 %, respectivement). Seulement la moyenne géométrique de la charge microfilarienne avait été réduite significativement (de 202,2 à 80,9 mf/mL). Il a cependant été mis en évidence une certaine hétérogénéité sur la réponse au traitement puisque sur les 116 individus microfilarémiques à l'inclusion, 15 sont devenus amicrofilarémiques, 69 ont gardé le même score ICT et 29 sont restés positifs mais ont diminué leur score de 1 point après 2 campagnes d'administration. Enfin, cette stratégie a également permis de réduire considérablement la prévalence de l'ankylostome, et dans une moindre mesure celle de la trichocéphalose et de l'ascaridiose (Pion et al. 2015).

Trois ans après l'instauration de cette stratégie, une nouvelle évaluation a été réalisée. Les prévalences de l'antigénémie et de la microfilarémie ont été réduites significativement. L'ankylostomose a disparu dès la deuxième année d'administration, la prévalence de l'ascaridiose a chuté drastiquement (77,2% de réduction) et celle de la trichocéphalose a également diminué (24,4% de réduction). Il est important de noter que la couverture thérapeutique était de plus de 80% à chaque campagne (Pion et al. 2017). Ces résultats ont permis de valider l'intérêt de la stratégie d'administration d'albendazole seul semi-annuelle pour combattre la filariose lymphatique dans les zones où la loase est coendémique mais a également permis de mettre en évidence que ces campagnes avaient un rôle important dans la lutte contre les géohelminthiases.

L'essai en République Démocratique du Congo a permis de valider ces résultats dans une population où les prévalences initiales étaient différentes : 31,6%, 12%, 58,6%, 14% et 4,1% pour l'antigénémie à *W. bancrofti*, la microfilarémie à *W. bancrofti*, l'ankylostomose, l'ascaridiose et la trichocéphalose, respectivement. La population a également été moins adhérente aux campagnes d'administration avec un taux d'observance entre 56% et 88%. Toutes les prévalences ont chuté significativement après 8 campagnes d'administration sauf pour l'ascaridiose (Pion et al. 2020).

Néanmoins, de manière similaire à Séké Pembe, une hétérogénéité importante a été observée dans l'élimination des infections au niveau individuel ; certains individus ont éliminé leurs infections rapidement, tandis que d'autres sont restés infectés après 8 campagnes d'administration. Afin de mieux comprendre ce phénomène, nous avons ré-analysé les données issues de ces deux essais communautaires afin d'évaluer l'effet de l'observance thérapeutique individuelle sur l'élimination des infections au niveau individuel.

Les parties 3.2.3 et 3.2.4 s'intéressent à l'effet de l'observance individuelle aux campagnes semi-annuelles d'administration d'albendazole sur la filariose lymphatique et sur les infections par géohelminthes. Pour évaluer l'effet de l'observance sur les infections, nous avons pris en compte les données longitudinales individuelles dans les analyses grâce à l'utilisation de modèles de survie paramétriques. Ces modèles permettent de modéliser le temps nécessaire à la réalisation d'un évènement qui, ici, sera la négativation des indicateurs d'infection (antigénémie et microfilarémie pour la filariose lymphatique et présence d'œufs dans les selles pour les trois géohelminthes).

A notre connaissance, il s'agit des premières études évaluant l'impact de l'adhérence individuelle aux programmes d'administration de masse d'albendazole sur le temps nécessaire pour éliminer ces infections, au niveau individuel.

### 3.2.3 Effet dose-réponse de l'administration d'albendazole seule sur la filariose lymphatique au niveau individuel

## Results from two cohort studies in Central Africa show that clearance of *Wuchereria bancrofti* infection after repeated rounds of mass drug administration with albendazole alone is closely linked to individual adherence

*Clinical Infectious Diseases*

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**Running tittle:** Lymphatic filariasis and albendazole

**Key point:** Longitudinal analysis of data from two community trials of mass drug administration with semi-annual albendazole on lymphatic filariasis demonstrated a clear dose-response treatment effect at individual level that underlines the importance of adherence for LF elimination programs.

### *3.2.3.1 Abstract.*

**Background**. Two community trials conducted from 2012 to 2018 in the Republic of Congo and the Democratic Republic of the Congo demonstrated the efficacy of semi-annual mass drug administration (MDA) with albendazole (ALB) alone on lymphatic filariasis (LF). However, a high inter-individual heterogeneity in the clearance of infection was observed.

**Methods**. We analyzed trial data to assess the effect of individual adherence to ALB MDA on clearance of circulating filarial antigenemia (CFA) and microfilaremia. Community residents were offered a single dose of ALB every 6 months and tested for LF with a rapid test for CFA at baseline and then annually. CFA test results were scored on a semi-quantitative scale. At each round, microfilaremia was assessed in CFA-positives. All CFA-positive subjects for whom at least one follow-up measure was available were included in the analyses. Parametric survival models were used to assess the influence of treatment adherence on LF infection indicators.

**Results**. Out of 2658 subjects enrolled in the trials, 394 and 129 were eligible for analysis of CFA and microfilaremia clearance, respectively. After adjusting for age, sex and initial CFA score, the predicted mean time for clearing CFA was shorter in persons who had taken 2 doses of ALB per year (3.9 years) than in persons who had taken 1 or 0 dose (4.4 and 5.3 years, $P < .001$ for both). A similar pattern was observed for microfilaremia clearance.

**Conclusions**. These results demonstrate a clear dose-response relationship for the effect of ALB on clearance of CFA and microfilaremia.

### *3.2.3.2 Introduction*

Lymphatic filariasis (LF) is a mosquito-borne parasitic infection caused mainly by *Wuchereria bancrofti.* The strategy for LF elimination is to interrupt the transmission cycle between humans and vectors. In African countries where onchocerciasis is endemic, programs provide annual mass drug administration (MDA) with ivermectin (IVM) plus albendazole (ALB). Bednets are also often provided to limit mosquito exposure. Treatment with IVM and ALB reduces the density of the larval stages of the parasite (microfilariae, Mf) in the blood. However, MDA has to be repeated for many years because these drugs have a limited efficacy for killing adult worms (World Health Organization 2000). In areas where LF is coendemic with loiasis, another filarial infection caused by *Loa loa*, this strategy is dangerous, because IVM can induce

serious adverse events (SAE) in people with very high *L. loa* microfilarial densities (MFD) (Gardon, Gardon-Wendel, Demanga-Ngangue, et al. 1997). In these areas, alternative strategies have to be implemented. Previous clinical trials comparing the effects of various drugs on *W. bancrofti* MFD suggested that treatment with ALB alone might reduce MFD, albeit at a slower rate than after combined treatment with IVM and ALB (Ismail et al. 2001; Makunde et al. 2003; Panicker et al. 1991; Dembele et al. 2010; Kazura 2010; Cartel et al. 1991, 1992; Fox et al. 2005; Gayen et al. 2013; Dunyo et al. 2000; Addiss et al. 1997). ALB does not induce SAEs in subjects with high *L. loa* MFD (Kamgno et al. 2016; Tabi et al. 2004; Tsague-Dongmo et al. 2002). In 2012, the World Health Organization (WHO) proposed that MDA with ALB (preferably semi-annual, and combined with integrated vector management) might be used to eliminate LF in areas where loiasis is coendemic (WHO 2012). The results of two community trials conducted in the Republic of Congo (Congo) and the Democratic Republic of the Congo (DRC) confirmed that this strategy was effective. In the first site, where baseline circulating filarial antigenemia (CFA) and Mf prevalences were moderate (17.3% and 5.3%, respectively), and treatment adherence was high (83-90%), these indicators decreased to 4.7% and to 0.3%, respectively, after three years of semi-annual MDA with ALB alone (Pion et al. 2017). In DRC, where baseline infection prevalences were higher and treatment adherence lower (56-88%), CFA and Mf prevalences decreased from 31.6% to 8.5% and from 12.0% to 0.9%, respectively, after four years of semi-annual MDA with ALB (Pion et al. 2020). Although MDA with ALB alone was highly effective at the community level, considerable heterogeneity was observed in parasite clearance at the individual level; some individuals cleared their infections rapidly, while others remained infected after 8 rounds of MDA. In this study, we have reanalyzed data collected from these two community trials to assess the effect of individual adherence to ALB treatment on the CFA and *W. bancrofti* microfilaremia clearance rates.

#### 3.2.3.3 *Methods*

### A. Study populations

The design of the two studies has been described elsewhere (Pion et al. 2017, 2020). In Congo, the study was conducted from 2012 to 2015 in Seke-Pembe, a village located in Mabombo Health District (Bouenza division). In DRC, the study site consisted of two contiguous villages (Mbunkimi and Misay) located in the Kwilu province, and the trial took place from 2014 to 2018.

Study participants were tested for LF infection at baseline and then annually. Both studies were approved by ethics committees and administrative authorities in the respective countries. Adult participants signed an informed consent form. Participants aged < 18 years were enrolled only after verbal assent and if one parent signed a consent form.

A total of 2658 individuals were examined for LF infection at least once during the two studies. The present analysis included all individuals who were CFA-positive at the time of their first test (which was not necessarily performed during the year when the trial started in the site) and who had at least one subsequent examination. Therefore, individuals who had progressed from CFA-negative to positive during the follow-up period and those who were CFA-negative at all time points tested were not included in the analysis.

## B. Assessment of *W. bancrofti* infection

Annual parasitological assessments were performed for participants aged ≥ 5 years. LF infections were detected by CFA testing using point-of-care tests. In Congo, testing was done with the BinaxNOW Filariasis immunochromatographic card test (ICT; Alere, Scarborough, ME, USA) in 2012, 2013 and 2014 and with the Filarial Test Strip (FTS; Alere, Scarborough, ME, USA) in 2015. All antigen testing in DRC was performed with FTS. ICT and FTS results were scored semi-quantitatively (0, 1, 2 or 3 according to the relative intensities of the test and control lines) (Pion et al. 2017; Chesnais et al. 2013). All CFA-positive individuals were invited to return for blood sampling between 10:00 PM and 1:00 AM for assessment of *W. bancrofti* microfilaremia. MFDs were based on the arithmetic mean of the counts of two 70-microliter thick blood smears and expressed as Mf per milliliter (Mf/mL).

## C. Drug distribution and assessment of treatment adherence

CFA-negative individuals were treated with a single tablet of ALB (400 mg) immediately after antigen testing under the direct observation of investigators. Those with positive CFA test results were treated with ALB just after collection of night blood for Mf testing. Residents who had not participated in the parasitological survey were also offered ALB treatment.

All treatments were provided under the supervision of a local healthcare worker who was also responsible for conducting a population census before each semi-annual MDA. Every treatment was recorded in a drug treatment register. In addition, during the annual assessment visits, we asked the participants if they had received ALB during the previous MDA campaign, six

months earlier. Therefore, for each year of the study, we could determine for each participant whether he/she had taken 2, 1 or 0 ALB tablets.

## D. Socio-demographics and risk factors for LF

At inclusion, we collected information about sex and age. At each visit, we also collected, using a standardized 1-page questionnaire, socio-demographic characteristics and habits that are known to be risk factors for LF such as bednet usage and occupation (fishing, hunting, farming and regular sleeping outside of the village in the bush) (Chesnais et al. 2019, 2014).

## E. Statistical analysis

The events analyzed are clearance of CFA (the transition from a positive to a negative CFA test during follow-up) and clearance of microfilaremia. We used survival analysis methods (Rodriguez 2010) to account for the individual follow-up nature of the data. The start date for the survival analysis was the first visit (index date). Individual observations were censored at the end of the follow-up or at the date of the event (date of the annual parasitological survey). Each participant's data were considered for calculation of cumulative person-years in the survival analysis.

We considered the following covariates for the analysis: sex, initial MFD (categorized in three categories of similar sample size: 1 to 150, 150 to 300 and > 300 Mf/mL), initial CFA score (from 1 to 3), a history of fishing as an occupation (yes or no) and a history of regularly sleeping in the bush (yes or no).

We also considered the following time-varying covariates: age (categorized according to interquartile and median values: 5-17, 18-30, 31-45 and $\geq$ 46 years old), number of ALB tablets taken during the previous year (0, 1 or 2), bednets use during the previous night (yes or no), the CFA test used (ICT or FTS).

Univariate analysis of clearance rates was conducted using Mantel-Haenszel tests. Clearance rates represents the probability of occurrence of clearance in a specified period of time.

We used a parametric survival models with accelerated failure time (Saikia and Pratim Barman 2017) to estimate the influence of time-varying variables on infection clearance (time-to-event)(Petersen 1986). Several time distributions that do not require meeting the proportional risk assumption were tested according to Akaike Information Criterion (AIC). For the survival models, random effects, at both village and household levels, were assessed using results

of likelihood-ratio tests. Results are presented as time ratios with 95% confidence intervals (95% CI). Time ratios represent time differences to event according to the reference category. Socio-demographic data, occupation, initial infection intensity (CFA and/or MFD) and the numbers of ALB tablets taken each year were included in the CFA and microfilaremia clearance survival models. The type of test (ICT or FTS) was included in the CFA clearance model. The fitted models used to estimate average times to clear CFA and microfilaremia included all explanatory variables.

A mixed model with random effect at individual level was used to describe changes in MFD according to time, treatment history and socio-demographic information. Several transformations (linear, quadratic, first-order fractional polynomials and second-order fractional polynomials) were tested for the time variable and selection was made according to AIC. As for CFA clearance analysis, random effects at village and household levels were assessed. Lastly, the significance of relevant interaction terms was assessed (age and sex, age and initial CFA score, age and initial MFD, age and number of ALB treatments taken, sex and initial CFA score, sex and initial MFD, sex and number of ALB treatments taken) for CFA and microfilaremia clearance and MFD change analyses. All analyses were performed using STATA v.15.1 software (StatCorps, LP, College Station, TX, USA).

#### *3.2.3.4 Results*

### A. Study participants

Out of the 2658 participants enrolled in the studies, 879 were only tested once; 22 who were CFA-negative at baseline acquired CFA (15 in DRC and 7 in Congo), and 1363 were CFA-negative at baseline and all follow-up times. Thus, observations from 394 participants were available for analysis of CFA clearance for a total of 1369 person-years of follow-up and 203 CFA clearance events. For the microfilaremia clearance analysis, 129 subjects had a total of 400 person-years of follow-up with 100 microfilaremia-clearance events. The survival data concerning non-time varying variables are summarized in Table 2.

### B. CFA and microfilaremia clearance rates

Clearance rates with significance values are presented in Table 3. The probability of CFA clearance was negatively correlated with initial CFA score and the probability of microfilaremia clearance was negatively correlated with initial MFD. A history of sleeping regularly in the bush decreased the probability of CFA clearance. CFA clearance was also more likely in the Congo site than in DRC. The probabilities for clearance of CFA for each of the 39 treatment patterns during the 5-year period are included in the Table 6.

## C. Parametric survival multivariate models for the clearance of CFA and microfilaremia

Results from the parametric survival model analyses are presented in Table 4. Log-logistic distribution and log-normal distribution were the best fits for time in the CFA and microfilaremia clearance models, respectively. No interactions between covariates were found. A random effect at village level ($P$ = .0277) was included in the CFA clearance model (Intraclass Correlation Coefficient = 7.27%), but this was not significant in the microfilaremia clearance model ($P$ = .346). CFA score at inclusion, frequent sleeping outdoors, and type of CFA test were all significantly associated with CFA clearance. Times to CFA clearance were significantly longer in individuals with higher initial CFA scores. Sleeping outdoors significantly increased the time to CFA clearance. Assessment of the CFA by ICT decreased the observed duration to CFA clearance. Predicted average time for clearing CFA was shorter in those who had taken two doses of ALB per year (3.9 years) than in those who has taken 1 or 0 dose (4.4 and 5.3 years, $P$ < .001 for both comparisons). Microfilaremia clearance had a similar pattern: individuals who had taken two doses of ALB per year became amicrofilaremic after a mean time of 3.1 years, whereas those who had taken 1 or 0 dose per year needed 3.6 ($P$ < .001) and 5.9 years ($P$ < .001), respectively, to clear their microfilaremia. Time to microfilaremia clearance was also significantly longer in individual with higher initial MFD.

## D. Changes in MFD over time

No transformation of time was required for the model. Neither the village- ($P$ = .496) nor the household-level ($P$ = .529) random effect was significant in the mixed model. Results from the mixed model with no random effect are presented in Table 5. MFD reduction was more rapid when individuals were adherent with MDA. The decrease in MFD was not significantly different in those who had taken 0 or 1 dose of ALB per year. Figure 12 shows predicted changes in MFD

according to the number of doses taken per year with time transformation into a fractional polynomial of order 2 (see Table 7). All predictions were adjusted for sex, age and initial MFD. Differences in slopes were highly significant between 0 and 2 doses of ALB (*P* = .009), between 2 and 1 dose (*P* = .004), but not significant between 1 and 0 dose (*P* = .419).

#### *3.2.3.5 Discussion*

The WHO's provisional recommendation to use semi-annual MDA with ALB alone to control LF in areas where *L. loa* is coendemic was based on thin evidence. The few trials (Addiss et al. 1997; Fox et al. 2005; Dunyo et al. 2000; Gayen et al. 2013) that had evaluated the effect of a single dose of ALB on LF infection had demonstrated a modest effect of the drug on MFD. In addition, two meta-analyses of the efficacy of a single dose of ALB alone on Mf and CFA prevalences concluded that this treatment would induce only a small to non-existent decrease in these outcomes at 3, 6, or 12 months post-treatment (Macfarlane et al. 2019; Addiss et al. 2005).

We conducted community trials in two settings to evaluate the impact of semi-annual MDA with ALB on LF (Pion et al. 2017, 2020). However, a major methodological limitation of these trials was that the effect of semi-annual treatment was not directly compared to that of annual treatment or no treatment. Thus, the analyses presented here provide important information regarding the added value of semi-annual ALB treatment vs annual MDA or no treatment on LF infection parameters. Our longitudinal analyses from infected persons clearly demonstrate a dose-response effect for ALB treatment on CFA and on microfilaremia. Our results show that good adherence leads to faster clearance of LF infection in individuals. Both clearances were significantly associated with the number of doses of ALB taken annually, and with initial infection levels. A lower initial CFA score was associated with a higher probability of CFA clearance. Therefore, the knowledge of the individual semi-quantitative results at baseline may be useful to improve planning for LF elimination programs. The use of the ICT test was associated with an increased probability of CFA clearance relative to use of the FTS, and this is likely due to the higher sensitivity of the FTS (Chesnais et al. 2017). Although baseline CFA scores were not associated with more rapid clearance of microfilaremia, higher initial MFD increased the time required for total microfilaremia clearance.

Regarding individuals' exposure and habits, individuals who sleep regularly outdoors took longer to clear CFA. This was probably due to reinfection, because prior studies have identified sleeping outdoors as a significant risk factor for LF in central Africa (Chesnais et al. 2014, 2019). However, the use of bednets or a history of fishing were not significantly associated with the clearances. Although the non-use of bednets has been shown to be a risk factor for LF infection, their use in infected individuals (without MDA) was not effective for clearing infections or for reducing Mf prevalence in the time frames (3 or 4 years) of this study (Richards et al. 2013). Data on the relationship between bednet usage and treatment adherence are included in the Table 8. Hunting and agricultural activities were not included in the models, because the numbers of hunters and farmers were small in the study sites, and inclusion of these occupations would have destabilized the models. Individuals were more likely to clear CFA after MDA in Congo than in DRC. This might be due to the fact that therapeutic coverage was higher and more constant and baseline infection prevalence lower, in the Congo site than in DRC which may reduce transmission and, therefore, the probability that re-infection occurs.

The activity of ALB alone for LF has important implications for current protocols that rely heavily on CFA surveys in school-aged children for MDA stopping decisions and post-MDA surveillance. Indeed, since soil-transmitted helminth programs routinely only treat children, using this demographic as a sentinel for MDA stopping decisions may underestimate the level of community-wide transmission because LF will tend to be less prevalent in children, who are more treated than adults.

We elected to use parametric survival models to analyze these data because these models are more flexible and allow longitudinal analyses with time-varying variables (i.e. ALB intake). In addition, they are more informative than non-parametric approaches, because they provide time ratios, enable predictions of mean and median survival times and have more power than semi-parametric models. Log-logistic distribution for the CFA clearance model and log-normal distribution for the microfilaremia clearance model were the best fits for our data, and they have the advantage of not requiring proportional risk assumptions, unlike conventional Cox survival models.

The presence of bias cannot be excluded. Prevalence bias may be present. However, fewer than 11% of the population (8.5 and 10.4% for the CFA and microfilaremia clearance models, respectively) had taken ALB prior to our study. We believe that this bias, if it exists, would have had very little impact on our results. In addition, participation bias cannot be excluded

either: people with a high participation frequency in our study may have different characteristics than non-participants, including adherence with treatment.

We have mentioned that participation rates in MDA decreased over time, probably reflecting a kind of fatigue on the part of some community members (Pion et al. 2020). We believe that we have demonstrated through these new analyses that participation rates in MDA programs must be maintained at high levels to accelerate the elimination of LF in individuals and communities. Evidence from this study could be used in social mobilization programs to illustrate the importance of achieving and sustaining high rates of MDA adherence in LF elimination programs.

| | | CFA clearance | | Microfilaremia clearance | |
|---|---|---|---|---|---|
| **Variables** | **Categories** | **PY [a]** | **Events [b]** | **PY [a]** | **Events [b]** |
| | | 1369 | 203 | 400 | 100 |
| Sex | Male | 754 | 112 | 232 | 61 |
| | Female | 615 | 91 | 168 | 39 |
| Age at inclusion | 5 – 17 years | 304 | 47 | 87 | 22 |
| | 18 – 30 years | 349 | 48 | 92 | 23 |
| | 31 – 45 years | 402 | 59 | 113 | 24 |
| | ≥ 46 years | 314 | 49 | 108 | 31 |
| CFA score at inclusion | 1 | 488 | 128 | 30 | 9 |
| | 2 | 352 | 51 | 91 | 28 |
| | 3 | 529 | 24 | 279 | 63 |
| Bednets use at inclusion | No | 561 | 82 | 138 | 33 |
| | Yes | 808 | 121 | 262 | 67 |
| Fishing activities at inclusion | No | 596 | 100 | 161 | 43 |
| | Yes | 699 | 95 | 211 | 52 |
| History of sleep outside at inclusion | No | 953 | 159 | 248 | 63 |
| | Yes | 408 | 44 | 144 | 36 |
| Village | Misay (DRC) | 297 | 39 | 85 | 21 |
| | Mbunkimi (DRC) | 660 | 79 | 199 | 49 |
| | Seke Pembe (Congo) | 412 | 85 | 116 | 30 |
| Study site | Bouenza (Congo) | 957 | 118 | 284 | 70 |
| | Kwilu (DRC) | 412 | 85 | 116 | 30 |
| Initial MFD | 0 – 150 Mf/mL | | | 144 | 44 |
| | 151 – 300 Mf/mL | | | 77 | 22 |
| | > 300 Mf/mL | | | 179 | 34 |

**Tableau 2. Survival data for time constant variables used in circulating filarial antigenemia (CFA) and microfilaremia survival models**

[a] Person-years

[b] Number of events.

| | | CFA | | | Microfilaremia | | |
|---|---|---|---|---|---|---|---|
| **Variables** | **Categories** | **Clearance rates [a]** | **95% CI [b]** | ***P* [c]** | **Clearance rates [a]** | **95% CI [b]** | ***P* [c]** |
| | | 14.8 | 12.9 – 17.0 | | 25.0 | 20.5 – 30.4 | |
| Sex | Male | 14.8 | 12.0 – 18.2 | .989 | 26.3 | 20.4 – 33.8 | .543 |
| | Female | 14.8 | 12.3 – 17.9 | | 23.2 | 17.0 – 31.8 | |
| Age at inclusion | 5 – 17 years | 15.5 | 11.6 – 20.6 | .859 | 25.3 | 16.6 – 38.4 | .739 |
| | 18 – 30 years | 13.7 | 10.4 – 18.2 | | 25.0 | 16.6 – 37.6 | |
| | 31 – 45 years | 14.7 | 11.4 – 18.9 | | 21.2 | 14.2 – 31.7 | |
| | ≥ 46 years | 15.6 | 11.8 – 20.6 | | 28.7 | 20.2 – 40.8 | |
| CFA score at inclusion | 1 | 26.2 | 22.1 – 31.2 | < .0001 | 30.0 | 15.6 – 57.6 | .184 |
| | 2 | 14.5 | 11.0 – 19.1 | | 30.8 | 21.2 – 44.6 | |
| | 3 | 4.5 | 3.0 – 6.8 | | 22.6 | 17.6 – 28.9 | |
| Bednets use at inclusion | No | 14.6 | 11.8 – 18.1 | .677 | 23.9 | 17.0 – 33.6 | .752 |
| | Yes | 14.9 | 12.5 – 17.9 | | 25.6 | 20.1 – 32.5 | |
| Fishing activity at inclusion | No | 16.8 | 13.8 – 20.4 | .671 | 19.0 | 14.1 – 25.6 | .444 |
| | Yes | 13.6 | 11.1 – 16.6 | | 16.2 | 12.4 – 21.3 | |
| History of sleep outside at inclusion | No | 16.7 | 14.3 – 19.5 | .042 | 26.7 | 19.8 – 36.0 | .696 |
| | Yes | 10.8 | 8.0 – 14.5 | | 24.6 | 18.8 – 32.2 | |
| Village | Misay (DRC) | 13.1 | 9.6 – 18.0 | < .0001 | 24.7 | 16.1 – 37.9 | .85 |
| | Mbunkimi (DRC) | 12.0 | 9.6 – 14.9 | | 24.6 | 18.6 – 32.6 | 9 |
| | Seke Pembe (Congo) | 20.6 | 16.7 – 25.5 | | 25.9 | 18.1 – 37.0 | |
| Study site | Bouenza (Congo) | 12.3 | 10.3 – 14.8 | < .001 | 24.6 | 19.5 – 31.1 | .82 |
| | Kwilu (DRC) | 20.6 | 16.7 – 25.5 | | 25.9 | 18.1 – 37.0 | 6 |
| Initial MFD | 1 – 150 Mf/mL | | | | 30.6 | 22.7 – 41.0 | .036 |
| | 151 – 300 Mf/mL | | | | 28.6 | 18.8 – 43.4 | |
| | > 300 Mf/mL | | | | 19.0 | 13.6 – 26.6 | |

**Tableau 3. Univariate clearance rates for circulating filarial antigenemia (CFA) and microfilaremia**

[a] Calculated for 100 PY.

[b] 95% confidence intervals.

[c] *P* is calculated from significance tests using Mantel-Haenszel method based on stratified rate ratios.

| | | CFA clearance | | | Microfilaremia clearance | | |
|---|---|---|---|---|---|---|---|
| **Variables** | **Categories** | **TR [a]** | **95% CI [b]** | ***P*** | **TR [a]** | **95% CI [b]** | ***P*** |
| Sex | Female | Ref. | | | Ref. | | |
| | Male | 1.01 | 0.96–1.06 | .718 | 0.94 | 0.84–1.05 | .281 |
| Age | 5 – 17 years | Ref. | | | Ref. | | |
| | 18 – 30 years | 1.00 | 0.93–1.07 | .910 | 1.02 | 0.87–1.20 | .769 |
| | 31 – 45 years | 1.02 | 0.95–1.08 | .515 | 1.15 | 0.98–1.34 | .082 |
| | ≥ 46 years | 1.00 | 0.93–1.07 | .992 | 1.03 | 0.89–1.20 | .644 |
| Initial CFA score | 1 | Ref. | | | Ref. | | |
| | 2 | 1.14 | 1.08–1.20 | < .001 | 0.91 | 0.74–1.11 | .357 |
| | 3 | 1.40 | 1.31–1.51 | < .001 | 1.00 | 0.83–1.21 | .982 |
| Annual treatment | 0 dose | 1.35 | 1.26–1.45 | < .001 | 1.82 | 1.46–2.27 | < .001 |
| | 1 dose | 1.12 | 1.06–1.19 | < .001 | 1.18 | 1.04–1.34 | .008 |
| | 2 doses | Ref. | | | Ref. | | |
| Bednets | No | Ref. | | | Ref. | | |
| | Yes | 0.98 | 0.94–1.03 | .515 | 0.94 | 0.84–1.04 | .243 |
| Fishing | No | Ref. | | | Ref. | | |
| | Yes | 0.97 | 0.92–1.02 | 0.280 | 1.06 | 0.95–1.18 | .305 |
| Sleep outside | No | Ref. | | | Ref. | | |
| | Yes | 1.09 | 1.03–1.16 | .002 | 1.05 | 0.94–1.18 | .397 |
| Test used | FTS | Ref. | | | | | |
| | ICT | 0.76 | 0.69–0.85 | < .001 | | | |
| Initial MFD | 1 – 150 Mf/mL | | | | Ref. | | |
| | 151 – 300 Mf/mL | | | | 1.04 | 0.92–1.19 | .514 |
| | > 300 Mf/mL | | | | 1.28 | 1.14–1.43 | <.001 |

**Tableau 4. Results from parametric survival models for CFA (with village as a random effect) and microfilaremia clearance**

[a] Adjusted time ratio
[b] 95% confidence intervals.

| Variables | Categories | Adjusted coefficients | 95% CI [a] | *P* |
|---|---|---|---|---|
| Sex | Female | Ref. | | |
| | Male | -26.6 | -131.2 – 77.9 | .618 |
| Age | 5 – 17 years | Ref. | | |
| | 18 – 30 years | -93.1 | -236.6 – 50.4 | .203 |
| | 31 – 45 years | 35.3 | -103.6 – 174.1 | .619 |
| | ≥ 46 years | -59.6 | -196.8 – 77.5 | .394 |
| Initial CFA score | 1 | Ref. | | |
| | 2 | -37.8 | -250.9 – 175.4 | .728 |
| | 3 | 52.6 | -156.8 – 262.0 | .622 |
| Initial MFD | 1 – 200 Mf/mL | Ref. | | |
| | > 200 Mf/mL | 221.3 | 124.3 – 318.3 | < .001 |
| Bednets | No | Ref. | | |
| | Yes | 29.9 | -61.9 – 121.8 | .523 |
| Fishing | No | Ref. | | |
| | Yes | -18.5 | -133.9 – 96.9 | .753 |
| Sleep outside | No | Ref. | | |
| | Yes | -21.0 | -138.5 – 96.5 | .753 |
| Annual treatment | 0 dose | Ref. | | |
| | 1 dose | 177.8 | -472.3 – 828.0 | .592 |
| | 2 doses | 416.6 | -217.3 – 1050.5 | .198 |
| Time | Continuous | -22.0 | -174.6 – 130.5 | .777 |
| Annual treatment interacted with time | 0 dose | Ref. | | |
| | 1 dose | -68.6 | -234.9 – 97.7 | .419 |
| | 2 doses | -210.9 | -369.3 – -52.44 | .009 |

**Tableau 5. Mixed model results for the evolution of Mf density (MFD).**

[a] 95% confidence intervals.

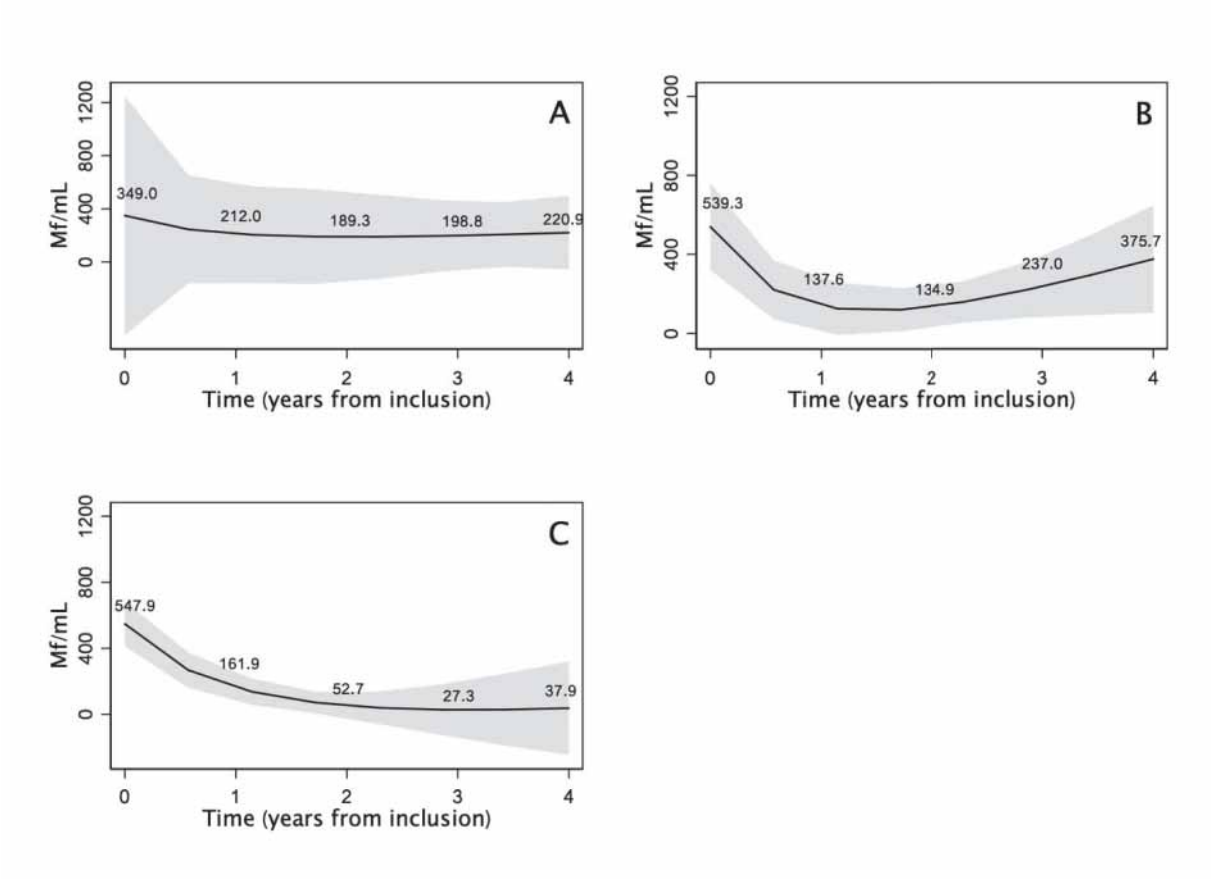


**Figure 12. Predictions of Mf densities (MFD) evolution according to time (fractional polynomial of order 2) and adherence with MDA (A: 0 dose per year, B: 1 dose per year; C: 2 doses per year).**

Full model and time transformations are available in Table 9.

| Duration of follow-up | Treatment Pattern [a] | N [b] | CFA clearance [c] | Proportion of CFA-negative subjects at the end of follow-up |
|---|---|---|---|---|
| 1 year | 0 | 5 | 2 | 40.0% |
| | 1 | 30 | 6 | 20.0% |
| | 2 | 54 | 15 | 27.8% |
| | **Total** | 89 | 23 | 25.8% |
| 2 years | 0 2 | 2 | 1 | 50.0% |
| | 1 0 | 1 | 1 | 100% |
| | 1 1 | 8 | 5 | 62.5% |
| | 1 2 | 12 | 8 | 66.7% |
| | 2 1 | 15 | 5 | 33.3% |
| | 2 2 | 52 | 30 | 57.7% |
| | **Total** | 90 | 50 | 55.6% |
| 3 years | 0 0 0 | 2 | 1 | 50.0% |
| | 0 2 0 | 1 | 1 | 100% |
| | 0 2 1 | 1 | 1 | 100% |
| | 1 0 2 | 1 | 0 | 0% |
| | 1 1 0 | 2 | 2 | 100% |
| | 1 1 2 | 1 | 1 | 100% |
| | 1 2 0 | 3 | 3 | 100% |
| | 1 2 1 | 2 | 1 | 50.0% |
| | 1 2 2 | 11 | 5 | 45.5% |
| | 2 0 1 | 1 | 0 | 0% |
| | 2 0 2 | 1 | 0 | 0% |
| | 2 1 1 | 3 | 1 | 33.3% |
| | 2 1 2 | 13 | 6 | 46.1% |
| | 2 2 0 | 14 | 14 | 100% |
| | 2 2 1 | 6 | 3 | 50% |
| | 2 2 2 | 88 | 51 | 57.9% |
| | **Total** | 150 | 90 | 60.0% |
| 4 years | 0 1 2 0 | 1 | 1 | 100% |
| | 0 2 1 2 | 1 | 1 | 100% |
| | 1 1 2 0 | 1 | 1 | 100% |
| | 1 1 2 2 | 1 | 0 | 0% |
| | 1 2 0 0 | 1 | 1 | 100% |
| | 1 2 2 2 | 6 | 2 | 33.3% |
| | 2 0 2 1 | 1 | 0 | 0% |
| | 2 1 2 0 | 1 | 1 | 100% |
| | 2 1 2 2 | 1 | 1 | 100% |
| | 2 2 0 0 | 4 | 4 | 100% |
| | 2 2 1 0 | 2 | 2 | 100% |
| | 2 2 2 0 | 8 | 8 | 100% |
| | 2 2 2 1 | 3 | 2 | 66.7% |
| | 2 2 2 2 | 34 | 16 | 47.0% |
| | **Total** | 65 | 40 | 61.5% |
| **Total** | | 394 | 203 | 51.5% |

**Tableau 6. Proportion of subjects who were CFA-negative at the end of their follow-up according to the number of albendazole doses received during each year of follow-up ("treatment pattern")**

[a] the first digit corresponds to the number of treatment received during the first year of follow-up, the second digit corresponds to the number of treatment received during the second year of follow-up, etc.

[b] numbers of subjects corresponding to each pattern

[c] numbers of subjects who experienced CFA negativation during the last year of follow-up.

| Variables | Categories | Adjusted coefficients | 95% CI [a] | *P* |
|---|---|---|---|---|
| Sex | Female | Ref. | | |
| | Male | -47.1 | -147.5 – 53.3 | .358 |
| Age | 5 – 17 years | Ref. | | |
| | 18 – 30 years | -68.5 | -206.4 – 69.5 | .331 |
| | 31 – 45 years | 28.6 | -103.7 – 161.0 | .672 |
| | ≥ 46 years | -60.4 | -191.8 – 70.9 | . 367 |
| Initial CFA score | 1 | Ref. | | |
| | 2 | -43.7 | -247.2 – 159.8 | .674 |
| | 3 | 56.1 | -145.0 – 257.3 | .584 |
| Initial MFD | 1 – 200 Mf/mL | Ref. | | |
| | > 200 Mf/mL | 175.8 | 81.6 – 270.0 | < .001 |
| Bednets | No | Ref. | | |
| | Yes | -11.0 | -100.9 – 78.9 | .810 |
| Fishing | No | Ref. | | |
| | Yes | 16.3 | -94.3 – 126.9 | .773 |
| Sleep outside | No | Ref. | | |
| | Yes | -31.7 | -143.2 – 79.7 | .577 |
| Annual treatment | 0 dose | Ref. | | |
| | 1 dose | -78.7 | -455.4 – 297.9 | .682 |
| | 2 doses | -62.5 | -427.3 – 302.3 | .737 |
| FP1 (Time)[b] | Continuous | -286.9 | -2221.3 – 1647.5 | .771 |
| FP2 (Time)[c] | Continuous | 128.8 | -806.3 – 1063.9 | .787 |
| Annual treatment interacted with FP1 (Time) | 0 dose | Ref. | | |
| | 1 dose | -654.1 | -2698.4 – 1390.3 | .531 |
| | 2 doses | -450.9 | -2417.8 – 1515.9 | .653 |
| Annual treatment interacted with FP2 (Time) | 0 dose | Ref. | | |
| | 1 dose | 392.7 | -631.7 – 1417.1 | .452 |
| | 2 doses | 132.6 | -841.5 – 1106.7 | .790 |

**Tableau 7. Model results for the evolution of Mf density (MFD) with time as a fractional polynomial of order 2.**

[a] 95% confidence intervals

[b] the transformation for the Fractional polynomial 1 is: log(time) - .7406723224

[c] the transformation for the Fractional polynomial 2 is: $\log(\text{time})^2$ - .5485954892

| Bed net usage | Treatment adherence N (%) | | | Total |
|---|---|---|---|---|
| | 0 dose in the year | 1 dose in the year | 2 doses in the year | |
| **No** | 60 (55.6%) | 109 (37.5%) | 253 (30.8%) | 422 (34.6%) |
| **Yes** | 48 (44.4%) | 182 (62.5%) | 569 (69.2%) | 799 (65.4%) |
| **Total** | 108 | 291 | 822 | 1221 |

Cuzick test: Z = 4.992, $P = 0.0001$

Spearman coefficient between bed net usage (yes/no) and treatment adherence (0, 1 or 2 doses) for all observations: 0.129 ($P < 0.0001$)

**Tableau 8. Relationship between bed nets use and treatment adherence for all observations included in parametric survival model on CFA clearance**

N: number of observations, %: percentage of observations according to bed net usage

### 3.2.4 Quantification de la valeur ajoutée de l'administration d'albendazole seule sur les infections par géohelminthes au niveau individuel

## A strong effect of individual compliance with mass drug administration for lymphatic filariasis on sustained clearance of soil-transmitted helminth infections

*Parasites & Vectors*

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**Keywords:** Soil-transmitted helminths, Albendazole, Parametric survival analysis, Treatment adherence, Mass drug administration

#### *3.2.4.1 Abstract*

**Background**: The impact of semi-annual mass drug administration (MDA) with albendazole alone (ALB 400 mg) on lymphatic filariasis (LF) and soil-transmitted helminth (STH) infections was assessed during two trials conducted from 2012 to 2018 in the Republic of Congo and Democratic Republic of Congo. The data collected were analyzed to evaluate the effect of compliance to ALB treatment on STH infections.

**Methods**: STH infections were diagnosed with duplicate Kato-Katz thick smears and results were reported as eggs per gram (EPG) of stool. All subjects with at least two STH infection assessments were included in the analyses. We used parametric survival models to assess the

influence of compliance to ALB treatment on the probability of (a) achieving sustained clearance of STH infection and (b) acquiring STH infection during the follow-up.

**Results**: Out of 2,658 subjects included in the trials, data on 202 persons (701 person-years, PY), 211 (651 PY) and 270 (1013 PY) with hookworm, *Ascaris lumbricoides* and *Trichuris trichiura* were available to analyze the probability of reaching sustained clearance of infection. The effect of ALB was dose-related for all three STH. For hookworm, the time required for sustained clearance was longer (4.3 years, $P < 0.001$) or shorter (3.4 years, $P = 0.112$) for participants who took 0 and 2 doses per year, respectively, compared to those who took 1 dose per year (3.7 years). For *Ascaris*, the time required to obtain a sustained clearance followed the same pattern: 6.1 years ($P < 0.001$) and 3.2 years ($P = 0.004$) versus 3.6 years for, 0, 2 and 1 dose per year, respectively. For *Trichuris*, compliant participants required less time for sustained clearance (4.2 years, $P < 0.001$) than those who took only 1 dose per year (5.0 years). ALB was more effective for achieving sustained clearance of STH infection in subjects with light baseline infection intensities compared to those with higher egg counts.

**Conclusion**: Our results illustrate the importance of MDA compliance at the level of the individual with respect to the STH benefit provided by semiannual ALB MDA that is used for LF elimination in Central Africa.

#### *3.2.4.2 Background*

Soil-transmitted helminth (STH) infections are among the most common infections in the world and affect poor and disadvantaged communities, particularly those living in sub-Saharan Africa, Southeast Asia and Latin America. In 2010, it was estimated that 438.9 million people were infected with hookworm, 819.0 million with *Ascaris lumbricoides* and 464.6 million with *Trichuris trichiura* (Pullan et al. 2014). The current strategy to control STH infections is to conduct periodical deworming campaigns without individual diagnosis that target at-risk populations in endemic areas (preschool children, school-age children, women of reproductive age and adults in certain high-risk occupations). The World Health Organization (WHO) recommends the use of benzimidazoles: albendazole (400 mg) or mebendazole (500 mg). Because of logistic and cost constraints, preventive chemotherapy campaigns for STH are usually conducted once or twice per year, depending on the initial prevalence of infection with any STH (World Health Organization 2012). However, the optimal frequency of administration to maximize impact

remains a topic of discussion (Anderson et al. 2012). In this study, we had the opportunity to assess the individual effect of a semi-annual albendazole (ALB) treatment on STH infections. The data originate from two community trials that were designed to evaluate the effect of mass drug administration (MDA) with semi-annual ALB on lymphatic filariasis (LF) in two countries in central Africa. The first study was conducted in a village in the Republic of the Congo (Congo), where *A. lumbricoides* infection prevalence decreased significantly from 56.5% to 12.9% and *T. trichiura* infection prevalence decreased significantly from 78.6% to 59.4% after 7 rounds of ALB MDA with global treatment adherence between 83 and 90% (Pion et al. 2017). The second study took place in two contiguous villages in the Democratic Republic of the Congo (DRC), where hookworm infection prevalence decreased significantly from 58.6% to 21.2% after 8 rounds of ALB MDA with a global treatment adherence between 56% and 88%; *Ascaris* and *Trichuris* infection prevalences also decreased (from 14.0% to 1,6% and from 4.1% to 2.9%, respectively) (Pion et al. 2020). The results of these two trials are consistent with what was previously observed regarding ALB efficacy: according to a meta-analysis of more than 50 clinical trials, ALB has very good efficacy for clearing hookworm infections, good efficacy for *Ascaris* infections, but only moderate efficacy for *Trichuris* infections (Moser et al. 2017).

Some studies have shown that semi-annual ALB MDA is highly effective for reducing STH prevalence at the community-level (Pion et al. 2015, 2017, 2020; Steinmann et al. 2015; Pullan et al. 2019; Vaz Nery et al. 2019). However considerable heterogeneity has been observed at the individual level, and no prior studies examined the impact of individual compliance with MDA on their STH infections. For example, in one study some individuals cleared their infections after a single treatment, while infections were still present in others (due to persistence or reinfection) after 8 rounds of semi-annual MDA (Cabada et al. 2014). In the present study, we used longitudinal treatment and parasitology data collected between 2012 and 2018 from two community MDA studies to assess relationships between MDA compliance by individuals with STH infections and their subsequent infection status. The results provide a clear link between MDA compliance by individuals and sustained clearance of STH infections.

#### *3.2.4.3 Methods*

### A. Study population

The design of the MDA studies has been described elsewhere (Pion et al. 2020, 2017). In Congo, the study was conducted from 2012 to 2015 in Seke-Pembe, a village located in Mabombo Health District (Bouenza division). In the DRC, the study site consisted of two neighbouring villages (Mbunkimi and Misay) located in the Kwilu province, and the trial took place from 2014 to 2018. Study participants were tested for STH infections at baseline and then annually. No deworming program had ever been conducted in the two areas prior to our trials. Both studies were approved by ethics committees and administrative authorities in the respective countries. Adult participants signed an informed consent form. Participants aged < 18 years were enrolled only after verbal assent and if one parent signed a consent form.

During the course of the two trials, a total of 2,658 individuals were examined at least once for LF and a total of 1,573 provided stool samples at least once.

## B. Assessment of STH infections

Annual parasitological assessments were performed for participants > 5 years of age. STH infections were detected by microscopic examination of stool specimens. Participants were given a 50 mL plastic stool container and asked to collect a sample of their stool in the morning. The stools specimens were collected and stored in cooling boxes and shipped within 6 hours to the laboratory where they were immediately processed or stored overnight at 6°C. Two thick smears were prepared according to the Kato-Katz method for each stool sample (Katz et al. 1972). Thick smears were examined by microscopy at 40x magnification, and the slides prepared from each sample were examined by two different microscopists. The arithmetic mean egg count from the two slides was calculated, and results were expressed for each species as eggs per gram of stool (EPG).

## C. Drug distribution and assessment of treatment adherence

All participants were offered treatment with one ALB tablet (400 mg) that was swallowed under the direct observation of study staff. All inhabitants who had not participated in the parasitological survey or who missed testing (due to absence or refusal) were later visited at home and offered ALB treatment. All treatments were provided under the supervision of a local healthcare worker who was also responsible for conducting a population census before each semi-annual MDA. Every treatment was recorded in a drug treatment register. In addition, participants were asked whether they had received ALB during the previous MDA campaign (six

months earlier) during annual parasitological surveys. Therefore, for each annual parasitological assessment, we determined if each participant had taken 2, 1 or 0 ALB treatments since the last parasitological assessment.

## D. Statistical analysis

The primary endpoint (i.e. event of interest) for the study was conversion in STH infection(s). This was considered separately for conversion from a positive to a negative test during follow-up (defined as the sustained clearance of infection analysis) and for the conversion from negative to positive (defined as the incident infection analysis).

The sustained clearance analysis included all individuals who were positive for *A. lumbricoides*, *T. trichiura* or hookworm at the time of their first test (which was not necessarily performed during the year when the trial started in the site) who also had at least one subsequent stool tested for STH. Therefore, individuals who were negative at baseline and remained negative throughout their follow-up were not included in the analyses (representing 59.1%, 47.8% and 37.6% of the population with at least one follow-up test for hookworm, *Ascaris* and *Trichuris*, respectively). The numbers of excluded individuals are provided in Table 9. A sensitivity analysis including all people who were positive at the time of their first test and who became negative during their follow-up, regardless of whether they subsequently became positive again is provided in Table 14.

| | **Hookworm infection** | | ***Ascaris* infection** | | ***Trichuris* infection** | |
|---|---|---|---|---|---|---|
| | **DRC** | **Congo** | **DRC** | **Congo** | **DRC** | **Congo** |
| **Number of participants included in the sustained clearance analysis** | **202** | | **211** | | **270** | |
| • Participants who had sustained clearance of their infections | 86 | 18 | 21 | 151 | 5 | 80 |
| • Participants who did not clear their infections | 98 | 0 | 6 | 33 | 3 | 182 |
| **Number of participants not included in the sustained clearance analysis** | **2456** | | **2447** | | **2388** | |
| • No follow-up (only 1 visit) | 911 | 1003 | 911 | 1003 | 911 | 1003 |
| • Negative at all points | 81 | 359 | 207 | 149 | 217 | 63 |
| • Progressed from negative to positive test | 101 | 1 | 132 | 45 | 141 | 53 |

**Tableau 9. Individuals included or not included in the sustained clearance analysis**

The incident infection analysis included all individuals who were negative for *A. lumbricoides*, *T. trichiura* or hookworm at the time of their first stool test who had a positive stool test at a later time point (regardless of whether they subsequently became negative again).

We used survival analysis methods for the sustained clearance and incident infection analyses. The start date for the survival analysis was the first visit (index date) with a positive STH test for the sustained clearance of infection analysis or a negative STH test for the incident infection analysis. Individual observations were censored at the end of the follow-up or at the date of the event (date of the annual parasitological survey). Each participant's data were considered for calculation of cumulative person-years in the survival analysis.

We considered the following non-time-varying covariates for each STH analysis: sex and initial EPG intensity (categorized according to WHO guidelines (Montresor et al. 1998)).

We also considered the following time-varying covariates: age (categorized according to interquartile and median values: 5-8, 9-12, 13-30 and $\geq$ 31 years old), and the number of ALB tablets taken during the previous year (0, 1 or 2).

Univariate analysis of infection status conversion rates was conducted using Mantel-Haenszel tests. We used parametric survival models with accelerated failure time to estimate the influence of time-varying variables on infection status conversion (time-to-event) (Saikia and Pratim Barman 2017; Petersen 1986). These models allow longitudinal analyses with time-varying variables; they are more informative and provide time ratios that enable prediction of mean time until an event occurs (either sustained clearance or incident infection events).

Several time distributions that do not require meeting the proportional risk assumption were tested according to Akaike Information Criterion (AIC). Random effects at the household and at the village level were assessed in all survival models, and significance was assessed using results of likelihood-ratio tests. Significant random effects were retained in the models. Results are presented as time ratios with 95% confidence intervals (95% CI). Time ratios represent time differences to event (individual infection status conversion) according to the reference category. Socio-demographic data, occupation, initial infection intensity and the numbers of ALB tablets taken per year were included in the *Ascaris*, *Trichuris* and hookworm infection status conversion survival models. For the *Trichuris* model, the variable "numbers of ALB tablets taken per year" had only two categories (1 or 2) because only 1 person-year contributed to the 0-dose category. Predicted average times to infection status conversions were estimated, using the command *margins* in STATA v.15.1 software (StatCorps, LP, College Station, TX, USA) (Williams 2012).

For individuals who achieved sustained clearance of infection, mixed models with random effects were used to describe changes in EPG according to time, treatment history and socio-demographic information for each STH infection. Several transformations (linear, quadratic, first-order fractional polynomials and second-order fractional polynomials) were tested for the time variable and selection was made according to AIC. Random effects at village level were considered for the parametric survival analysis and mixed models for changes in EPG. Lastly, the significance of relevant interaction terms was assessed (age and sex, age and initial infection intensity, age and initial infection intensity, age and number of ALB treatments taken, sex and initial infection intensity, sex and initial infection intensity, sex and number of ALB treatments taken) for all models. All analyses were performed using STATA v.15.1 software.

#### *3.2.4.4 Results*

### A. Study participants

For the sustained clearance model, 202 of 2,658 participants enrolled in the studies (7.6%) had repeated observations for the hookworm infection analysis with 701 person-years (PY) of observation; 135 persons (66.8% of individuals diagnosed with hookworm infection at their first parasitological exam) had negative stool examinations for hookworm during the course of the study and remained so until their last follow-up visit. For *Ascaris*, 211 (7.9%) participants had longitudinal data for analysis with 681 PY of observation and 172 (81.5% of individuals with *Ascaris* at their first parasitological exam) became negative during the course of the study and remained so until their last follow-up visit. For *Trichuris*, 270 (10.2% of all study participants) had baseline and follow-up data for the analysis with 1,019 PY of observation and 85 (31.5% of individuals with *Trichuris* infection at their first parasitological exam) became negative during the course of the study and remained so until their last follow-up visit. The key infection survival data are summarized in Table 10 along with results of a bivariate analysis of cofactors. Of note, most individuals with hookworm infection lived in the DRC study site whereas most individuals with *Ascaris* and *Trichuris* infections lived in the Congo study site.

For the incident infection model, out of 542 individuals negative for hookworm at baseline with at least one follow-up visit, 102 (220 PY) experienced an incident infection (18.8%). For *Ascaris*, out of 533 participants negative at baseline with at least one follow-up visit, 177 (727

PY) experienced an incident infection (33.2%). For *Trichuris*, out of 474 participants negative at baseline with at least one follow-up visit, 194 (772 PY) experienced an incident infection (40.9%).

## B. Bivariate analysis of sustained clearance of STH infection

The unadjusted sustained clearance rates for hookworm, *Ascaris* and *Trichuris* infections were 19.2, 25.2, and 8.4 events per 100 PY of observation, respectively (Table 10). Table 10 also shows sustained clearance rates adjusted for each covariable. Because the number of ALB tablets taken per year is a time-varying variable, it was not included in the sustained clearance rate calculations. For hookworm and *Ascaris*, sustained clearance occurred with a higher probability in females than in males. Older individuals converted to negative more often than children for all three STH infections. Despite low numbers of people with heavy worm loads in early years of the study, initial intensity of infection was negatively correlated with the probability of sustained clearance for *Ascaris* and *Trichuris*. Finally, the probability of sustained clearance varied by village of residence.

## C. Parametric survival multivariate models for sustained clearance of soil-transmitted helminths infection

Parametric survival model results for sustained clearance are presented in Table 11. For the hookworm and *Ascaris* models, a log-normal distribution gave the best fit to the data whereas a log-logistic distribution was a better fit for *Trichuris*. No significant interactions between the covariates were found. Household random-effects were included for the *Trichuris* model (Intraclass Correlation Coefficient [ICC] was 12.2%, $P = 0.002$). Village random-effects were included for the hookworm model (ICC was 18.0%, $P < 0.001$). Random effects were not significant for the *Ascaris* model. For hookworm, *Ascaris* and *Trichuris*, older individuals (over 30 years of age) achieved sustained clearance more rapidly than children aged 5 to 8 years (3.3 vs 5.7 year for hookworm; 3.1 vs 3.8 year for *Ascaris* and 4.1 vs 4.7 year for *Trichuris*). Non-compliant individuals (0 doses per year) took significantly longer to achieve sustained clearance for *Ascaris* or hookworm than those who took one dose per year (6.1 vs. 3.6 years for *Ascaris* [$P < 0.001$] and 4.3 vs. 3.7 years for hookworm [$P < 0.001$], respectively). In addition, individuals who were highly compliant with MDA (2 doses per year) took less time to achieve sustained clearance for *Ascaris* and *Trichuris* infection than individuals who took only one dose per year (3.2 vs. 3.6 years for *Ascaris* [$P = 0.004$] and 4.2 vs. 5.0 years for *Trichuris* [$P < 0.001$], respectively).

Figure 14 illustrates that, for all three STH infections, the predicted proportion of people who experienced sustained clearance of infection was higher in highly compliant individuals and increased with the duration of follow-up. Figure 14 also shows that in individuals with low compliance, sustained clearance was observed after two and three years of follow-up for hookworm and *Ascaris* infection, respectively, whereas it started after one year in fully-compliant individuals.

Turning to infection intensity, individuals with moderate to heavy initial infection intensity with *Trichuris* (≥ 1000 EPG) took significantly longer to achieve sustained clearance than individuals with light infections (< 1000 EPG) (4.2 vs. 5.0 years, respectively). Baseline infection intensities did not significantly affect the time to sustained clearance for *Ascaris* or hookworm infections.

## D. Parametrical survival models of STH incident infection

Parametric survival model results for *incident infection* are presented in Table 11. A log-logistic distribution gave the best fit for data in the hookworm and *Trichuris* models; a log-normal distribution provided the best fit for the *Ascaris* model. No interactions between the covariates were found. Household random-effects were included for the *Trichuris* model (ICC was 42.9%, $P < 0.001$). Village random-effects were included for the *Ascaris* models (ICC was 45.7%, $P < 0.001$) and hookworm model (ICC was 31.4%, $P < 0.001$). For hookworm and *Trichuris*, males acquired infection significantly slower than females. For *Trichuris* model, older individuals (> 13 years) acquired infection significantly faster than individuals aged 5 to 8 years. Non-compliant individuals (0 doses per year) took significantly less time to acquire *Ascaris* infection than those who took one dose of ALB per year.

## E. Changes in EPG over time

Linear transformation of time provided the best fit for these data. No multilevel effects were included in these mixed models, because neither the village nor the household effect was significant. Overall decreases in EPG were superior in individuals with better compliance (Table 13). For all three STH, the decline in EPG over time was slower in young individuals, regardless of the number of ALB tablets taken per year.

According to the mixed model, decreases in hookworm EPG were not significantly different for individuals with different compliance patterns: - 81.2 EPG/year (95% CI: -316.0 –

153.6) for 1 dose and -197.1 EPG/year (CI 95%: -402.6 – 8.4) for 2 doses, compared with no treatment. Decreases in *Ascaris* EPG were significantly different between 0 dose and 1 dose per year (regression coefficient: -7,076.1 EPG/year, CI 95%: -13,103 – -1,048.9) and between 0 dose and 2 doses (regression coefficient: -6,207.3 EPG/year, CI 95%: -1.2196 – -218.5). Finally, decreases in *Trichuris* EPG were not significantly different for individuals who took 2 doses per year (regression coefficient: -207.5 EPG/year, CI 95%: -603.2–188.3), compared to those who took 1 dose per year.

| | | Hookworm (202 individuals) | | | | | *Ascaris* (211 individuals) | | | | | *Trichuris* (270 individuals) | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Variables** | **Categories** | **PY [a]** | **N Events [b]** | **Rate [c]** | **95% CI [d]** | ***P* [e]** | **PY [a]** | **N events [b]** | **Rate [c]** | **95% CI [d]** | ***P* [e]** | **PY [a]** | **N events [b]** | **Rate [c]** | **95% CI [d]** | ***P* [e]** |
| | All participants | 701 | 135 | 19.2 | 16.2 – 22.8 | | 681 | 172 | 25.2 | 21.7 – 29.3 | | 1019 | 86 | 8.4 | 6.8 – 10.4 | |
| Sex | Male | 390 | 69 | 17.7 | 14.0 – 22.4 | 0.023 | 299 | 63 | 21.1 | 16.5 – 27.0 | 0.017 | 420 | 36 | 8.6 | 6.2 – 11.9 | 0.919 |
| | Female | 311 | 66 | 21.2 | 16.7 – 27.9 | | 382 | 109 | 28.5 | 23.7 – 34.4 | | 599 | 50 | 8.3 | 6.3 – 11.0 | |
| Age | 5 – 8 years | 245 | 30 | 12.2 | 8.6 – 17.5 | 0.009 | 153 | 20 | 13.1 | 8.4 – 20.3 | 0.001 | 201 | 7 | 3.5 | 1.7 – 7.3 | <0.001 |
| | 8 – 12 years | 166 | 39 | 23.5 | 17.2 – 32.2 | | 189 | 46 | 24.3 | 18.2 – 32.5 | | 222 | 9 | 4.0 | 2.1 – 7.8 | |
| | 13 – 30 years | 135 | 24 | 17.8 | 11.9 – 26.5 | | 117 | 32 | 27.3 | 19.3 – 38.7 | | 209 | 16 | 7.6 | 4.7 – 12.5 | |
| | 31 years and more | 155 | 42 | 27.1 | 20.0 – 36.6 | | 222 | 74 | 33.3 | 26.5 – 41.9 | | 387 | 54 | 13.9 | 10.7 – 18.2 | |
| Initial | Light | 575 | 131 | 20.0 | 16.8 – 23.7 | 0.059 | 269 | 77 | 28.6 | 22.9 – 35.8 | 0.022 | 634 | 74 | 11.7 | 9.3 – 14.7 | <0.001 |
| infection | Moderate | 29 | 4 | 13.8 | 5.2 – 36.5 | | 374 | 87 | 23.3 | 18.8 – 28.7 | | 369 | 11 | 3.0 | 1.6 – 5.4 | |
| intensity [f] | Heavy | 16 | 0 | 0 | N/A | | 38 | 8 | 21.0 | 10.5 – 42.1 | | 16 | 1 | 6.2 | 0.9 – 44.4 | |
| Village | Misay | 323 | 61 | 18.9 | 14.7 – 24.3 | 0.001 | 44 | 8 | 18.2 | 9.1 – 36.3 | 0.247 | 3 | 1 | N/A | N/A | |
| | Mbunkimi | 336 | 56 | 16.7 | 12.8 – 21.6 | | 41 | 13 | 31.7 | 18.4 – 54.6 | | 20 | 4 | N/A | N/A | |
| | Seke Pembe | 42 | 18 | 42.8 | 27.0 – 68.0 | | 596 | 151 | 25.3 | 21.6 – 29.7 | | 994 | 80 | 8.0 | 6.5 – 10.0 | |

**Tableau 10. Sustained clearance rates for hookworm, Ascaris and Trichuris infections**

[a] Person-years (PY)

[b] Number of events

[c] Infection status conversion rate (for 100 PY)

[d] 95% confidence intervals

[e] *P* is calculated from significance tests using Mantel-Haenszel method based on stratified rate ratios.

[f] According to WHO guidelines

- For hookworm: 1-1999, 2000-3999, more than 4000 Eggs Per Gram (EPG)
- For *Ascaris*: 1-4999, 5000-49999, more than 49999 EPG.
- For *Trichuris*: 1-999, 1000-9999, more than 10000 EPG.

| Variables | Categories | Hookworm | | *Ascaris* | | *Trichuris* | |
|---|---|---|---|---|---|---|---|
| | | TR / 95% CI [a] | *P* | TR / 95% CI [a] | *P* | TR / 95% CI [a] | *P* |
| Sex | Female | Ref. | | Ref. | | Ref. | |
| | Male | 1.10 [1.01,1.19] | 0.020 | 1.11 [1.02,1.21] | 0.013 | 0.99 [0.92,1.06] | 0.807 |
| Age | 5 – 8 years | Ref. | | Ref. | | Ref. | |
| | 8 – 12 years | 0.93 [0.83,1.04] | 0.223 | 0.95 [0.86,1.08] | 0.398 | 1.06 [0.91,1.23] | 0.452 |
| | 13 – 30 years | 0.87 [0.77,0.98] | 0.019 | 0.86 [0.75,1.00] | 0.046 | 0.96 [0.83,1.10] | 0.557 |
| | More than 30 years | 0.79 [0.70,0.88] | <0.001 | 0.81 [0.71,0.92] | 0.001 | 0.88 [0.77,0.99] | 0.049 |
| Initial infection intensity [b] | Light | Ref. | | Ref. | | Ref. | |
| | Moderate to heavy | 1.20 [0.98,1.46] | 0.072 | 1.06 [0.97,1.16] | 0.170 | 1.18 [1.07,1.31] | <0.001 |
| Treatment | 0 dose / year | 1.16 [1.00,1.35] | <0.001 | 1.74 [1.28,2.38] | <0.001 | Not calculable | |
| | 1 dose / year | Ref. | | Ref. | | Ref. | |
| | 2 doses / year | 0.91 [0.81,1.02] | 0.112 | 0.87 [0.79,0.95] | 0.004 | 0.84 [0.77,0.91] | <0.001 |
| Random effects | | Village | <0.001 | Not included | | Household | 0.002 |
| | ICC | 18.0% | | | | 12.2% | |
| Model | Distribution | Log-normal | | Log-normal | | Log-logistic | |
| | AIC | 464.1 | | 515.9 | | 410.2 | |
| | Log likelihood | -222.0 | | -249.0 | | -196.1 | |

**Tableau 11. Results from parametric survival models for sustained clearance of hookworm, Ascaris and Trichuris infections (with random effect)**

[a] Adjusted Time Ratio / 95% Confidence intervals. For example, for the *Ascaris* model, compared to the female (TR = 1), a male will take 11% (TR = 1.11) more time to achieve sustained clearance.

[b] According to OMS guidelines (light, moderate + heavy)

- For hookworm: 1-1999, more than 2000 Eggs Per Gram (EPG)
- For *Ascaris*: 1-4999, more than 5000 EPG.
- For *Trichuris*: 1-999, more than 1000 EPG.

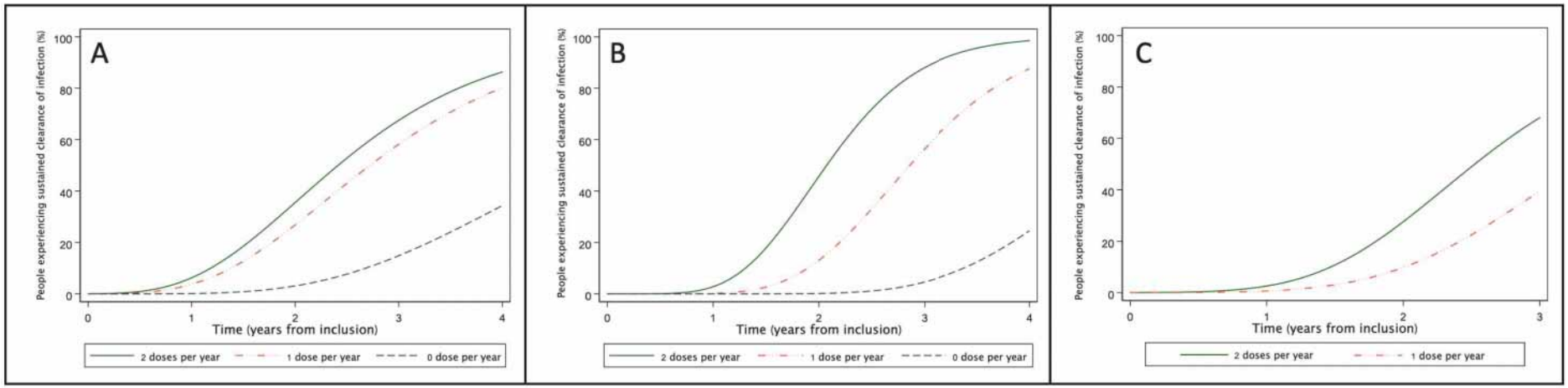


**Figure 13. The influence of compliance with albendazole treatment on soil-transmitted helminths infection status in persons with prior infections**

(A) Hookworm; (B) *Ascaris* and (C) *Trichuris*.

| Variables | Categories | Hookworm | | *Ascaris* | | *Trichuris* | |
|---|---|---|---|---|---|---|---|
| | | **TR / 95% CI [a]** | ***P*** | **TR / 95% CI [a]** | ***P*** | **TR / 95% CI [a]** | ***P*** |
| Sex | Female | Ref. | | Ref. | | Ref. | |
| | Male | 1.39 [1.11,1.73] | 0.004 | 1.10 [0.94,1.19] | 0.234 | 1.22 [1.031,1.44] | 0.018 |
| Age | 5 – 8 years | Ref. | | Ref. | | Ref. | |
| | 8 – 12 years | 1.12 [0.87,1.45] | 0.361 | 1.07 [0.88,1.32] | 0.489 | 0.88 [0.73,1.05] | 0.156 |
| | 13 – 30 years | 1.02 [0.72,1.46] | 0.872 | 1.13 [0.87,1.46] | 0.364 | 0.62 [0.46,0.84] | 0.002 |
| | More than 30 years | 0.95 [0.70,1.28] | 0.738 | 1.11 [0.87,1.41] | 0.412 | 0.53 [0.40,0.70] | <0.001 |
| Treatment | 0 dose / year | 0.79 [0.51,1.22] | 0.290 | 0.62 [0.42,0.91] | 0.001 | 0.78 [0.53,1.13] | 0.190 |
| | 1 dose / year | Ref. | | Ref. | | Ref. | |
| | 2 doses / year | 0.97 [0.70,1.33] | 0.828 | 1.10 [0.80,1.50] | 0.559 | 1.14 [0.85,1.54] | 0.385 |
| Random effects | | Village | < 0.001 | Village | < 0.001 | Household | <0.001 |
| | ICC | 31.4% | | 45.7% | | 42.9% | |
| Model | Distribution | Log-logistic | | Log-normal | | Log-logistic | |
| | AIC | 505.5 | | 626.5 | | 718.4 | |
| | Log likelihood | -243.7 | | -304.2 | | -350.2 | |
| Key survival data | Number of subjects | 102 | | 177 | | 194 | |
| | Person-years | 420 | | 727 | | 772 | |

**Tableau 12. Results from parametric survival models for incident hookworm, Ascaris and Trichuris infections (with random effect)**

[a] Adjusted Time Ratio / 95% Confidence intervals. For example, for the Hookworm model, incidence in males (TR = 1.39) is 39% slower than in females (TR = 1).

| Variables | Categories | Hookworm | | *Ascaris* | | *Trichuris* | |
|---|---|---|---|---|---|---|---|
| | | **Coeff. / 95% CI [a]** | ***P*** | **Coeff. / 95% CI [a]** | ***P*** | **Coeff. / 95% CI [a]** | ***P*** |
| Sex | Female | Ref. | | Ref. | | Ref. | |
| | Male | 65.8 [-34.4,166.0] | 0.199 | 1056.7 [-1115.9,3229.2] | 0.340 | 234.2 [-139.1,607.5] | 0.219 |
| Age | 5 – 8 years | Ref. | | Ref. | | Ref. | |
| | 8 – 12 years | 32.6 [-122.6,187.8] | 0.681 | -4540.2 [ -7576.5, -1504.0] | 0.003 | 126.1 [-439.3, 691.6] | 0.662 |
| | 13 – 30 years | -128.0 [-274.1,18.0] | 0.086 | -6432.5[ -9910.3, -2954.8] | 0.0001 | -333.9 [ -929.6, 261.7] | 0.272 |
| | More than 30 years | -107.1 [-251.4,37.1] | 0.088 | -8030.3 [-11035, -5024.5] | <0.001 | -498.6 [-1179.8, -162.3] | 0.010 |
| Initial infection intensity [b] | Light | Ref. | | Ref. | | Ref. | |
| | Moderate | 1155.4 [929.3,1383,5] | <0.001 | 8727.8 [6302.6, 11153] | <0.001 | 1061.2 [653.1, 1469.4] | <0.001 |
| | Heavy | 3171.6 [2875.8,3467.3] | <0.001 | 33843 [28637, 39050] | <0.001 | 6886.7 [5518.5, 8254.9] | <0.001 |
| Time [c] | Continuous | -58.2 [-263.2,146.6] | 0.577 | 1631.5 [-4174.5, 7437.4] | 0.582 | -91.9 [-378.8, 195.0] | 0.530 |
| Annual treatment interacted with time [c] | 0 dose | Ref. | | Ref. | | Not calculable | |
| | 1 dose | -81.2 [-316.0,153.6] | 0.498 | -7076.1 [-13103, -1048.9] | 0.021 | Ref. | |
| | 2 doses | -197.1 [-402.6,8.4] | 0.060 | -6207.3 [-12196, -218.5] | 0.042 | -207.5 [-603.2, 188.3] | 0.304 |
| Study site | Bandundu | Ref. | | Ref. | | Ref. | |
| | Seke Pembe | -196.9 [-427.8,34.0] | 0.095 | 2596.8 [-1004.8, 6198.3] | 0.158 | 721.0 [-410.1, 1852.1] | 0.212 |
| Intercept at baseline | 0 dose | 387.4 [-478.5,1253.2] | 0.381 | -7884 [ -33495, 17725.7] | 0.546 | Not calculable | |
| | 1 dose | 272.7 [-636.0,1181.4] | 0.556 | 22598 [-3365.0, 48561] | 0.088 | -199.5 [-170.9, 1305.9] | 0.795 |
| | 2 doses | 404.0 [-439.6,1247.7] | 0.348 | 22888 [-2750.4, 48527] | 0.080 | 936.6 [ -192.0, 2065.1] | 0.104 |

**Tableau 13. Mixed model results for the evolution of EPG intensity**

[a] Adjusted regression coefficient / 95% Confidence intervals.

[b] According to OMS guidelines

- For hookworm: 1-1999, 2000-3999, more than 4000 Eggs Per Gram (EPG)
- For *Ascaris*: 1-4999, 5000-49999, more than 49999 EPG.
- For *Trichuris*: 1-999, 1000-9999, more than 10000 EPG.

[c] Interpretation of the interaction variable: for hookworm model, all else being equal, each participants EPG decreased by 58.2 each year; and all else being equal, participants taking 1 dose and 2 doses pear year, as compared to 0 dose decreased their EPG by 81.2 and 197.1 EPG per year, respectively.

| Variables | Categories | Hookworm (209 individuals) | | *Ascaris* (222 individuals) | | *Trichuris* (285 individuals) | |
|---|---|---|---|---|---|---|---|
| | | TR / 95% CI [a] | *P* | TR / 95% CI [a] | *P* | TR / 95% CI [a] | *P* |
| Sex | Female | Ref. | | Ref. | | Ref. | |
| | Male | 1.12 [1.03,1.21] | 0.005 | 1.10 [1.01,1.19] | 0.022 | 0.98 [0.91,1.06] | 0.665 |
| Age | 5 – 8 years | Ref. | | Ref. | | Ref. | |
| | 8 – 12 years | 0.98 [0.88,1.09] | 0.741 | 0.96 [0.84,1.08] | 0.499 | 1.06 [0.92,1.23] | 0.397 |
| | 13 – 30 years | 0.92 [0.80,1.05] | 0.210 | 0.89 [0.77,1.02] | 0.091 | 0.99 [0.86,1.14] | 0.867 |
| | More than 30 years | 0.82 [0.71,0.95] | 0.009 | 0.84 [0.74,0.95] | 0.006 | 0.88 [0.77,0.99] | 0.043 |
| Initial infection intensity [b] | Light | Ref. | | Ref. | | Ref. | |
| | Moderate to heavy | 1.08 [0.91,1.28] | 0.396 | 1.08 [1.00,1.18] | 0.060 | 1.17 [1.06,1.29] | 0.002 |
| Treatment | 0 dose / year | 1.19 [1.02,1.38] | 0.026 | 1.77 [1.29,2.41] | <0.001 | Not calculable | |
| | 1 dose / year | Ref. | | Ref. | | Ref. | |
| | 2 doses / year | 0.93 [0.82,1.04] | 0.212 | 0.86 [0.79,0.95] | 0.002 | 0.80 [0.74,0.86] | <0.001 |
| Random effects | | Household | 0.001 | Not included | | Household | 0.004 |
| | ICC | 15.2% | | | | 11.9% | |
| Model | Distribution | Log-normal | | Log-normal | | Log-logistic | |
| | AIC | 494.2 | | 546.0 | | 477.2 | |
| | Log likelihood | -233.1 | | -264.0 | | -229.6 | |

**Tableau 14. Sensitivity analysis including people who were positive at the time of their inclusion and who sequentially changed to negative and to positive again during their follow-up**

[a] Adjusted Time Ratio / 95% Confidence intervals. For example, for the Hookworm model, compared females (TR = 1), Males took 12% (TR = 1.12) longer to achieve sustained clearance of infection. .

[b] According to OMS guidelines (light, moderate + heavy)

- For hookworm: 1-1999, more than 2000 Eggs Per Gram (EPG)
- For *Ascaris*: 1-4999, more than 5000 EPG.
- For *Trichuris*: 1-999, more than 1000 EPG.

#### *3.2.4.5 Discussion*

ALB is widely known to be effective for treatment of STH infections. Community MDA with ALB can reduce *Ascaris* and hookworm prevalence, but effects on *Trichuris* tend to be modest (Clarke et al. 2019; Moser et al. 2017). This is the first longitudinal study to examine the effect of individual compliance with semi-annual MDA with albendazole alone given for LF elimination on STH infections. We found that good compliance with semi-annual rounds of MDA resulted in shorter times to achieve sustained clearance of STH infections. This is likely due to a combination of curing existing infections and curing incident infections during the follow-up period. This dose-related effect was particularly strong for ascariasis. A similar pattern was observed for hookworm, although the difference between 1 and 2 doses per year was not statistically significant. This might indicate that reinfection is more rapid for hookworm than for *Ascaris*. Further studies are needed to validate this hypothesis. Individuals with *Trichuris* infection in this study were generally compliant with MDA, and this did not permit us to compare times to infection status conversion based on ALB intake. It is interesting that some non-compliant individuals with hookworm or *Ascaris* infections achieved sustained clearance, and this phenomenon was more common in the later years of the study, as shown in Figure 13. Although some spontaneous loss of infection is expected, particularly for light infections, an increase in sustained clearance events in later years might be due to a reduced force of infection in study communities (a “herd treatment effect”) as a result of MDA.

The use of random effects at the household level improved the parametric survival model for *Trichuris*. Continued infection in households due to non-compliance or shared poor sanitation might increase the risk of reinfection for other household members who comply with treatment. This would tend to increase mean times for sustained clearance of infection. Additional studies will be needed to test this hypothesis and to understand why the same household effect was not seen with hookworm or *Ascaris* infection.

It is interesting that incident infections were observed for all three STH infections during the course of the study despite community MDA. While most of these were probably true incidence events, it is likely that some of these were examples of pseudoincidence that may occur if light infections were not detected in prior stool samples.

Based on our analysis, good compliance with MDA is most effective for preventing *Ascaris* infections. That is because random household effects were significant for *Trichuris* incidence model and for the hookworm incidence model. This means that a fraction of the incident infections for hookworm and *Trichuris* are due to random household effects regardless of MDA compliance. Random effects set at the village level were significant for the hookworm sustained clearance model and the *Ascaris* incident infection model. This suggests that a significant fraction of these conversions is due to common village specific effects related to sanitation and/or infection-promoting behavior.

Higher infection intensity was negatively associated with sustained clearance for all three STH, but this only resulted in a significantly longer time to achieve sustained clearance for *Trichuris*. However, low baseline frequencies of moderate or high infection intensities for hookworm and *Ascaris* provided low statistical power for assessing the effect of infection intensity for those infections. For all models, shorter time to sustained clearance was associated with older age. This would be consistent with higher reinfection rates in children due to their behavior and exposure relative to those in older people (e.g., more close contact with other children, walking barefoot outdoors, and hand to soil to mouth).

We used mixed models to study changes in infection intensity (EPG) over time according to the number of ALB tablets taken per year for each STH. Only the *Ascaris* model showed a greater decrease in EPG for compliant participants. No significant differences were found in EPG decrease and ALB compliance in hookworm and *Trichuris* models. This is probably due to a lack of power, because EPG trended down in regression models as compliance increases.

One limitation of this study is that results may have been influenced by prevalence or participation bias. We believe that prevalence bias is unlikely to have impacted our results, because fewer than 20% of the study populations had taken ALB prior to our study. On the other hand, participation bias cannot be excluded, because people with a high participation frequency in our study may have had different characteristics (including MDA adherence) than non-participants.

As previously reported, MDA and stool survey participation rates decreased over time in these community MDA studies (Pion et al. 2020). This was probably due to study fatigue on the part of some community members. Results from this study that show clear benefits of compliance with MDA may help social mobilization campaigns to increase initial and sustained MDA adherence by individuals and populations.

### 3.2.5 Conclusions

**Ces deux études ont démontré une relation dose-réponse claire entre l'adhésion individuelle au traitement de masse semestriel par albendazole et (i) la disparition durable individuelle des infections à géohelminthes au fil du temps, (ii) la disparition durable individuelle de l'antigénémie circulante de la filariose lymphatique et (iii) la disparition durable individuelle de la microfilarémie de la filariose lymphatique.**

**Les personnes ayant une bonne compliance aux traitements de masse sont plus susceptibles de parvenir plus rapidement à une élimination durable des infections par la filariose lymphatique et géohelminthes. Ces résultats soulignent l'importance pour les programmes d'élimination de la filariose lymphatique d'atteindre et de maintenir des taux élevés d'observance thérapeutique. Ils peuvent être utilisés dans les campagnes de mobilisation sociale pour faire savoir à la population que le traitement de masse semestriel avec l'albendazole utilisée pour l'élimination de la filariose lymphatique apporte également une contribution très significative au contrôle des infections à géohelminthes, à la fois dans les communautés, et à l'échelle individuelle.**

**Ces résultats permettent également de répondre à certaines critiques faites aux essais communautaires évaluant l'albendazole comme traitement de masse. En effet, nous pensons que nos résultats vont permettre de faire réévaluer l'intérêt de l'utilisation biannuelle d'albendazole seul dans la prise en charge communautaire de la filariose lymphatique en répondant à certaines critiques émanant de la revue Cochrane (Macfarlane et al. 2019) comme :**

- **L'absence de données individuelles : grâce à notre plan d'analyse statistique, nous répondons complètement à cette critique et mettons en évidence que l'observance thérapeutique a une importance capitale dans la mise en place de ces programmes de lutte.**
- **Pas de groupe contrôle : nous répondons en partie à cette critique grâce à l'utilisation des données individuelles au cours du temps qui permettent de créer un groupe contrôle « théorique » d'individus qui ne prendraient jamais le traitement (groupe 0 dose par an).**

# 4. L’ivermectine, évaluation d’un effet prophylactique et étude systématique de ses effets indésirables graves

## 4.1 Généralités et historique de l'ivermectine

L'ivermectine est dérivée des avermectines (figure 14). Elle a été isolée à partir de la fermentation de *Streptomyces avermitilis*.

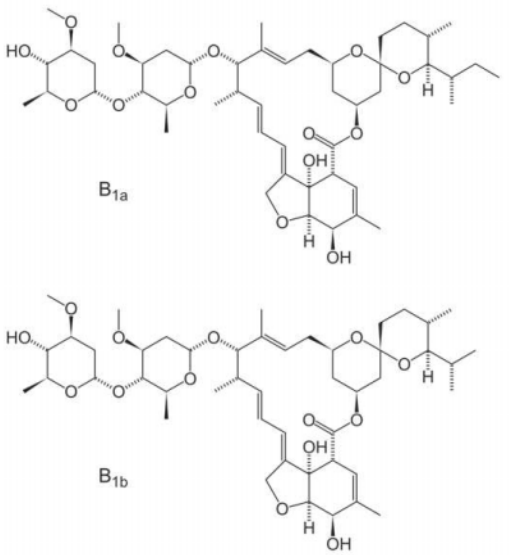


**Figure 14. Structure chimique de l'ivermectine**

Sa découverte dans les années 80 par Satochi Ômura, William C. Campbell et Tu Youyou leur a valu le prix Nobel de Physiologie et de Médecine en 2015. Cette nouvelle molécule antihelminthique a permis d'envisager l'élimination des nématodoses comme l'onchocercose et la filariose lymphatique.

## 4.2 Mécanismes d'action et indications de l'ivermectine

L'action antihelminthique de l'ivermectine est liée à sa capacité à inhiber la neurotransmission chez les vers grâce à son affinité importante pour les canaux chlorures glutamate-dépendants présents dans les cellules nerveuses et musculaires des invertébrés.

L'ivermectine est indiquée dans le traitement individuel de l'anguillulose, de la gale sarcoptique et de la filariose lymphatique. Comme indiqué précédemment, l'ivermectine est également utilisé dans les programmes de lutte contre la filariose lymphatique et contre l'onchocercose.

Concernant son action sur l'onchocercose, l'ivermectine possède deux actions principales lors d'une prise unique : une action microfilaricide, c'est-à-dire une action sur les microfilaires dermiques et une action embryostatique, c'est-à-dire le blocage de la production de microfilaires par les vers adultes. Enfin, lors de traitements rapprochés, l'ivermectine est capable d'agir indirectement sur l'embryogenèse des vers, c'est-à-dire une perturbation de l'insémination des adultes femelles.

L'ivermectine possède également deux actions que l'on considère comme mineures : une action macrofilaricide, c'est-à-dire la réduction de la longévité des vers adultes (en cas de traitements rapprochés) et une action prophylactique, c'est à dire un effet sur les stades larvaires pré-adultes des vers appelés stades L3 et stades L4. En réalité, cette action prophylactique n'a que très peu été étudiée chez l'homme, seulement 4 études *in vivo* ont été réalisé (Bain and Babayan 2003; Tchakouté et al. 1999; Boussinesq and Chippaux 2001; Njongmeta et al. 2004). Les stades L3 et L4 se développent lors des premiers mois suivant la piqûre d'une simulie infectée.

Nous avons réutilisé des données issues d'un essai clinique randomisé contrôlé évaluant l'effet macrofilaricide de l'ivermectine selon sa dose et sa fréquence d'administration. L'essai clinique initial s'est déroulé de 1994 à 1998 sur 643 individus. Grâce à ces données, nous avons pu évaluer l'effet prophylactique (c'est-à-dire un effet sur les stades larvaires et les femelles juvéniles d'*Onchocerca volvulus*) de quatre schémas d'administration d'ivermectine : 150 µg/kg par an, 800 µg/kg par an, 150 µg/kg tous les 3 mois et 800 µg/kg tous les 3 mois. L'effet prophylactique a été évalué par le biais d'un proxy : l'apparition et la disparition des nodules. Les analyses ont consisté à mesurer la différence de nodules apparus et disparus entre les 4 groupes de traitement après 3 ans de traitement. Par la suite, un modèle de régression de Poisson a été construit afin d'expliquer le nombre de nodules apparus lors de l'essai selon le groupe de traitement, le nombre de nodules initial et les variables sociodémographiques des patients.

Ces analyses permettent de comparer, pour la première fois, l'effet de différents schémas d'administration d'ivermectine sur l'apparition de nodules dans un contexte d'exposition à l'onchocercose afin de mettre en avant une éventuelle posologie ou fréquence d'administration pouvant avoir un effet prophylactique potentiel. Si l'augmentation de la fréquence d'administration permet d'obtenir un effet prophylactique plus important, il serait envisageable d'étudier la mise en place une chimioprophylaxie pour les expatriés et les voyageurs dans les zones connues pour être endémiques à l'onchocercose.

## 4.3 Effet prophylactique de l'ivermectine sur les stades larvaires d'*O. volvulus*

### Individuals living in an onchocerciasis focus and treated three-monthly with ivermectin develop fewer new onchocercal nodules than individuals treated annually

*Parasites & Vectors*

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### 4.3.1 Abstract

**Background**. Little information is available on the effect of ivermectin on the third- and fourth-stage larvae of *Onchocerca volvulus*. To assess a possible prophylactic effect of ivermectin on this parasite, we compared the effects of different ivermectin regimens on the acquisition of onchocercal nodules.

**Methods**. We analyzed data from a controlled randomized clinical trial of ivermectin conducted in the Mbam Valley (Cameroon) between 1994 and 1998 in a cohort of onchocerciasis infected individuals. The number of nodules that appeared between the start and the end of the clinical trial was analyzed, using ANOVA and multivariable Poisson regressions, between four treatment arms: 150 µg/kg annually, 800 µg/kg annually, 150 µg/kg 3-monthly, and 800 µg/kg 3-monthly.

**Results**. The mean number of nodules that appeared during the trial was reduced by 17.7% in subjects treated 3-monthly compared to those treated annually (regardless of the dose). Poisson regression model, adjusting on subject's age and weight, initial number of nodules and intensity of *O. volvulus* infection in his village of residence, confirmed that the incidence of new nodules was reduced in 3-monthly treatment arms compared to annually treatment arms, and that the dosage of ivermectin does not seem to influence this effect. Furthermore, the number of newly acquired nodules was positively associated with the initial number of nodules. Analysis of disappearance of nodules did not show any significant difference between the treatment groups.

**Conclusions.** To our knowledge, these results suggest for the first time in humans, that ivermectin has a partial prophylactic effect on *O. volvulus*. Three-monthly treatment seems more effective than annual treatment to prevent the appearance of nodules.

### 4.3.2 Background

Human onchocerciasis, also called "river blindness", is a neglected tropical disease (NTD) caused by the filarial nematode *Onchocerca volvulus*. In 1995, the World Health Organization (WHO) launched the African Programme for Onchocerciasis Control (APOC), which was mainly based on mass drug administration (MDA) of ivermectin (IVM). IVM, which is usually given annually at the standard dose of 150 µg/kg of body weight, has a direct effect on the microfilariae (mf) present in the skin (microfilaricidal effect) and prevents the release of new mf by the adult

female worms for several months (embryostatic effect). However, the effect of IVM on the viability of the adult worms (macrofilaricidal effect) is considered as moderate and treatments have to be repeated every year (or at shorter intervals) to maintain skin microfilarial densities (MFD) at low levels not associated with clinical manifestations. Besides this, little information is available on the effect of IVM on the third and fourth stage larvae (L3s and L4s) which develop to the adult stage during the first months following the bite of an infective black fly, and on the immature adults. The effect on these L3s, L4s, and immature adults, which would prevent the development up to the stage of fecund adult worms releasing mf, has been called causal prophylaxis, or suppressive effect (Lämmler 1977). We will use the term "prophylactic effect" throughout the text below.

Only four *in vivo* studies were conducted to evaluate the prophylactic effect of IVM on *Onchocerca* sp. The first one included 18 chimpanzees experimentally infected with *O. volvulus*. Six animals were treated with IVM (at 200 µg/kg) on the day of inoculation of the L3s, 6 were treated on day 28 and 6 were not treated. After having followed up the development of infection by repeated skin biopsies for 30 months, the authors concluded that IVM could have a partial effect on the L3s (which live for about two to three days in the definitive host before molting to the L4 stage (Bain and Babayan 2003)), but no effect on the L4s (Taylor et al. 1988). The second study was conducted in an area of North Cameroon where the cattle parasite *O. ochengi* is endemic. Two groups of calves between 2 and 8 weeks of age were treated monthly with subcutaneous IVM (Ivomec®) at either 200 µg/kg or 500 µg/kg for 21 months, and a third group was left untreated. Before each treatment, the animals were palpated for *O. ochengi* nodules and underwent a skin biopsy. The fact that none of the 15 treated calves developed adult worm infection, whereas 5 of the 6 control calves became infected led the authors to conclude that IVM had an effect on the L3s and L4s of *O. ochengi* (Tchakouté et al. 1999). The third study was conducted in an onchocerciasis hyperendemic focus (Mbam valley, Cameroon) and included subjects with no *O. volvulus* mf in skin biopsies ("skin snips" taken with a 2mm Holth punch). These subjects were treated, just after the start of the high transmission period, with either a single oral dose of IVM (150 µg/kg) plus ferrous sulphate tablets, or the latter drug only. One year after, the incidence of *O. volvulus* microfilaridermia was 23.4% in the IVM group and 25.8% in the control group, and the mean MFD were similar in the two groups (2.2 and 2.7 mf per skin snip, respectively). The authors concluded that a single dose of IVM had no perceptible prophylactic effect in this highly endemic area (Boussinesq and Chippaux 2001). The fourth study

included calves (mean age: 9 weeks) naturally exposed to *O*. *ochengi* infection, and treated with IVM at monthly or 3-monthly intervals, or left untreated. After 22 months of exposure, 11 of the 14 control animals had acquired nodules (including 10 with skin mf), two of the 10 animals treated 3-monthly had nodules (but no skin mf), and none of the 10 animals treated at monthly interval had acquired nodules (Njongmeta et al. 2004). These results suggest that 3-monthly treatment has a partial prophylactic effect on *O. ochengi*. Besides these trials, the effect of IVM on L3s and the L3-L4 molting process was supported by *in vitro* studies using *Onchocerca lienalis* (Court et al. 1985; Lok et al. 1987).

A double-blind randomized controlled trial aimed at assessing the potential macrofilaricidal effect of high (400-800 µg/kg) and/or more frequent (3-monthly) doses of IVM on *O. volvulus* was conducted in the Mbam valley (Cameroon) between 1994 and 1998. This effect was evaluated by the histologic examination of sections of nodules collected at the outset and at the end of the trial (Gardon et al. 2002). The proportion of dead female worms was found to be higher in the nodules collected from subjects treated 3-monthly than in those treated annually. During this trial, a careful examination for all palpable nodules was conducted at the outset of the trial, and during the nodulectomy round organized in 1997. The number and the location of each palpated nodule was noted on a standard chart. In the present paper, we present the results of statistical analyses performed on the number of nodules which had appeared or disappeared between the two examination rounds. Our main objective was to assess whether high doses or more frequent IVM treatment was associated with a lower number of new nodules (suggesting a prophylactic effect). Analyses were also performed on the number of nodules that had spontaneously disappeared.

## 4.3.3 Methods

### *4.3.3.1 Study population and subjects*

The protocol of the trial has been described in details elsewhere (Gardon et al. 2002). Briefly, it was conducted in the Bafia health district, located in the onchocerciasis hyperendemic focus of the Mbam valley (Cameroon). Eligible subjects were males aged 18-60 years in a good

state of health, with no contra-indication to IVM, and who presented at least two palpable nodules at the outset of the trial.

#### *4.3.3.2 Procedures*

After having signed an informed consent form, subjects were randomly allocated to one of the four treatment groups receiving either 150 µg/kg annually (control group), or high dose (400 µg, then 800 µg/kg) annually, or 150 µg/kg 3-monthly or high dose 3-monthly. The pre-treatment nodulectomy round was performed in May-June 1994. Before the nodulectomy, each participant was carefully examined and the location of each palpable nodule was noted on a standard anatomic chart. The randomly selected nodule to remove during the operation was represented by a green dot on the chart, and all the others were noted as a red dot. After nodulectomy, each participant received a 150 µg/kg "clearing dose" of IVM to avoid the possibility of severe reactions developing in any patients subsequently taking their first dose on the high-dose regimen. The 3-year courses of treatment under investigation began in August, 1994, 2–3 months after the clearing dose. A total of 643 subjects participated in this treatment round. The second round of nodulectomy was organized in August 1997. A total of 102 subjects was lost between August 1994 and August 1997 (24 deaths, 17 excluded on medical grounds and 61 subjects who moved away or were excluded because they missed one treatment round). The number of subjects participating in the second nodulectomy round was thus 541. Before the collection of the nodules, each patient was reexamined and the location of the nodules present was noted on the anatomic chart used in 1994. The nodules which had spontaneously disappeared in the interval were noted, and the location of the nodules which had appeared between 1994 and 1997 (thereafter called "new nodules") were noted by a blue dot. As these examinations had been performed just before the operation, an additional clinical examination was performed in November 1997, i.e. in a less time-constrained context, to confirm the results obtained three months before. This examination could be performed in 485 subjects and the statistical analyses were conducted on the latter.The study protocol has been approved by the Ethical Committee of Cameroon.

#### *4.3.3.3 Statistical Analysis*

Variables of interest were defined as (a) the number of nodules which appeared between 1994 and 1997 and (b) the number of nodules which disappeared. For these two variables, we used the same statistical analysis plan (Figure 15). Firstly, we assessed the difference between people treated annually and people treated 3-monthly, regardless of the dose, using a Student test. Then, comparisons were performed between the four treatment arms using an ANOVA. A Bonferroni test was subsequently used to assess which treatment arm(s) differed from the others. In case of ANOVA-associated P-value < 0.250, we performed a multivariable analysis to assess the possible associations between appearance of new nodules and the following variables: treatment group as categorical variable (firstly considering annual arms versus 3-monthly arms, then considering the four treatment arms), subject's age (expressed as continuous variable), subject's weight (continuous variable), initial number of palpable nodules (expressed sequentially as a continuous variable and as a categorical variable using the following categories: <5 nodules and ≥5 nodules), subject's initial microfilarial density (MFD, expressed as the number of mf per milligram of skin), and the intensity of *O. volvulus* infection in the participant's village of residence (proxy of exposure to infection). The individual MFD was calculated as the ratio of the sum of the number of mf found in both snips (taken from the left and right iliac crests) to the sum of the weights of each snip. In the analyses, the MFD was expressed as a categorical variable using the interquartile categories: <30, 31-80, 81-170, and >170 mf/mg. The intensity of *O. volvulus* infection in the participant's village of residence was expressed in three categories (low, moderate and high intensity) and was defined according to the community microfilarial load (CMFL) in the community measured during previous parasitological surveys in the community itself, or in neighbouring communities located at the same distance from the Mbam River. The CMFL, a classical indicator used to express the intensity of infection with *O. volvulus* in a community, corresponds to the Williams' geometric mean of the individual MFDs (here expressed as number of mf per skin snip) in all subjects examined (not only those skin snip positive) aged ≥20 years (Remme et al. 1986). The three categories used in the analyses correspond to CMFL < 20 mf/skin snip, [20 – 50] mf/skin snip and ≥ 50 mf/skin snip. Multivariable logistic regression models were used with the dependent variable coded as a binary outcome (appearance of nodules versus no appearance of nodule). The reference group is defined as

people with less than 5 nodules in the 150 µg/kg 3-monthly treatment group. Then, Poisson regression models were used, with the dependent variable recoded as 0, 1, 2, 3, 4 and ≥5 new nodules. We constructed two models: one with the 4 treatment arms and one with the pooled annual and 3-monthly treatment arms, regardless of the dose. For all models, an interaction term between the treatment arm and the initial number of nodules was added to assess the mean number of new nodules according to these two variables simultaneously. For each regression model, we presented two procedures: a saturated model with all explicative variables, whatever their *P*-values and a backward stepwise procedure with a *P*-value threshold of 0.150 for the explicative variables. We presented regression coefficients and their confidence intervals at 95% (95% CI). The reference group is defined as people who had the lowest number of initial nodules (i.e. 2 nodules) in the 150 µg/kg annually group or in the annual treatment, depending on the model. According to this regression analysis, predictions were made using the *margins* and *marginsplot* commands.

All analyses were performed using the STATA v.15.1 software (StatCorps, LP, College Station, TX, USA).

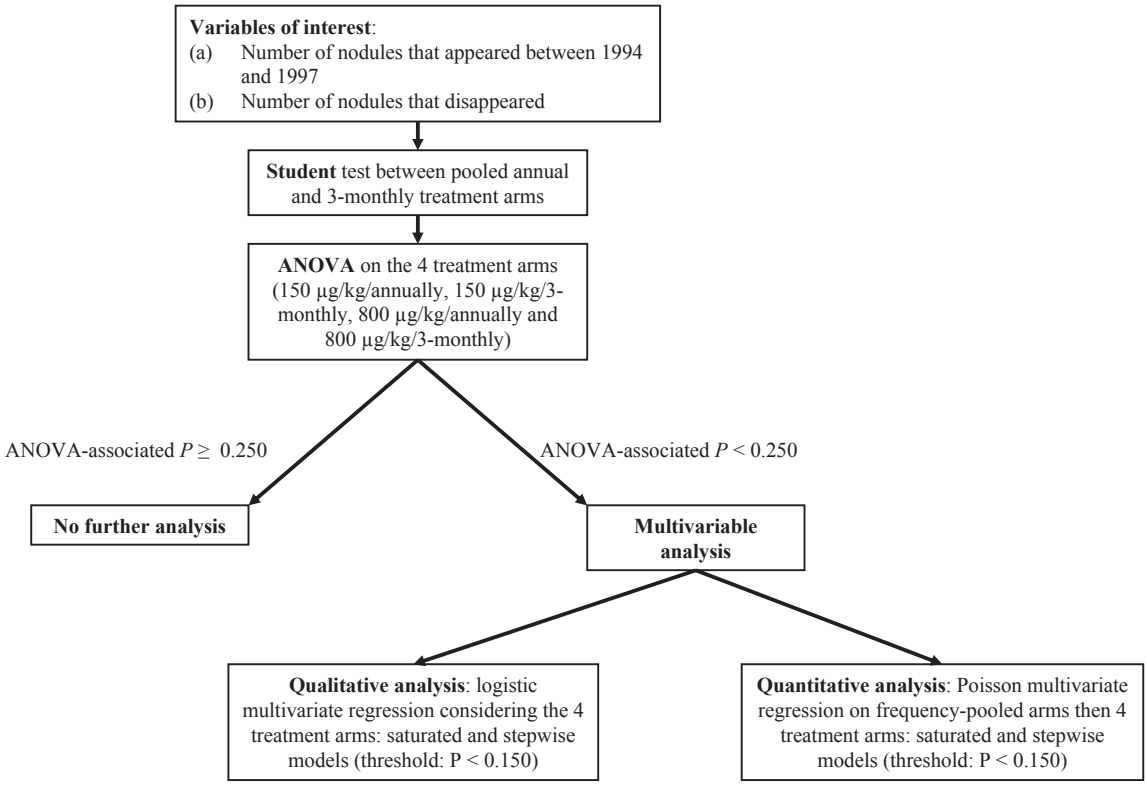


**Figure 15. Statistical analysis plan**

## 4.3.4 Results

### *4.3.4.1 Baseline characteristics*

Table 15 presents the baseline characteristics of the 485 study subjects as a whole and in each treatment arm. Before the first nodulectomy round, the participants of the four treatment groups were similar in terms of age (*P* = 0.714), body weight (*P* = 0.172), mean number of nodules (*P* = 0.349), CMFL in the village of residence (*P* = 0.574) and MFD (*P* = 0.450).

| | All participants | 150 µg/kg annually | 800 µg/kg annually | 150 µg/kg 3-monthly | 800 µg/kg 3-monthly |
|---|---|---|---|---|---|
| No. of subjects | 485 | 126 | 122 | 125 | 112 |
| < | 36.9 (12.0) | 36.4 (11.4) | 38.1 (12.4) | 36.7 (11.9) | 36.6 (12.3) |
| Mean weight (kg) (SD) | 63.0 (8.0) | 62.7 (8.2) | 64.3 (7.1) | 62.4 (8.5) | 62.7 (8.0) |
| Mean no. of nodules in 1994 (SD) | 5.7 (2.8) | 5.5 (2.5) | 5.8 (2.3) | 5.6 (3.2) | 5.9 (2.9) |
| Median no. of nodules in 1994 (IQR) | 5 (4-7) | 5 (4-7) | 6 (4-7) | 5 (3-7) | 6 (4-7) |
| CMFL in the village of residence: | | | | | |
| • Low (N, %) | 50 (10.3%) | 10 (7.9%) | 12 (9.8%) | 18 (14.4%) | 10 (8.9%) |
| • Middle (N, %) | 135 (27.8%) | 39 (31.0%) | 37 (30.3%) | 30 (24.0%) | 29 (25.9%) |
| • High (N, %) | 300 (61.9%) | 77 (61.1%) | 73 (59.8%) | 77 (61.6%) | 73 (65.2%) |
| Geometric mean of MFD<br>Median of MFD (IQR) | 56,0<br>79 (27-171) | 64,7<br>91 (34-197) | 50,8<br>71 (26-157) | 48,7<br>74 (24-143) | 61,8<br>84 (28-187) |

**Tableau 15. Baseline characteristics of the subjects included in 1994, before the start of the clinical trial**

No: number; SD: standard deviation; IQR: interquartile range; N: number of subjects; GM: geometric mean; CMFL: community microfilarial load; MFD: individual microfilarial density (mf/mg)

### *4.3.4.2 Analysis of appearance of nodules between 1994 and 1997*

The mean number of new nodules in the 237 subjects treated 3-monthly (1.85) was 17.7% lower than in the 248 subjects treated annually (2.25; Student test: *P* = 0.008). The mean numbers of nodules that appeared between 1994 and 1997 in subjects of each treatment group are shown in Table 16. ANOVA indicated a statistically significant difference in the number of new nodules between the treatment groups (*P* = 0.014). Bonferroni correction showed that the number of new nodules was significantly lower in the group which had received 150 µg/kg 3-monthly than in the group treated with 800 µg/kg annually (*P* = 0.008). No significant difference was found between the two groups treated annually (*P* = 1.000), nor between the two groups

treated 3-monthly (*P* = 0.871), nor between the two groups treated with 150 µg/kg (annually vs 3-monthly) (*P* = 0.260).

| | All participants | 150 µg/kg annually | 800 µg/kg annually | 150 µg/kg 3-monthly | 800 µg/kg 3-monthly | *P* |
|---|---|---|---|---|---|---|
| No. subjects | 485 | 126 | 122 | 125 | 112 | |
| Mean no. of new nodules (SD) | 2.1 (1.7) | 2.1 (1.7) | 2.4 (1.8) | 1.7 (1.5) | 2.0 (1.6) | 0.014 |
| Mean no. of nodules that disappeared (SD) | 0.5 (0.8) | 0.5 (0.8) | 0.5 (0.7) | 0.6 (0.8) | 0.6 (0.8) | 0.279 |

**Tableau 16. Appearance and disappearance of the nodules after 3 years of treatment**

no.: number; SD: standard deviation

According to the logistic regression analysis (Table 17), the probability to develop new nodules did not differ significantly between the treatment groups of subjects with less than 5 palpable nodules. In addition, people with more than 5 nodules and belonging to the groups treated annually with 150 µg/kg and 800 µg/kg had, respectively, 4.1 and 5.5 more chance to have new nodule(s) than those treated with 150 µg/kg 3-monthly and with less than 5 nodules. The CMFL in the village of residence and the individual MFD were not found to be associated with the probability of appearance of new nodules.

Table 18 shows the results of the Poisson regression model for each frequency of treatment explaining the count of new nodules. It shows that 3-monthly treatment (regression coefficient = 0.026; 95% CI = [0.005-0.048]), whatever the dose, is more than twice as effective as annual treatment (regression coefficient = 0.058 [0.035-0.081]) to prevent the appearance of nodules. The difference in the slope is significant (*P* = 0.001). Figure 16 represents the predictions of this model. Table 19 shows the results of the Poisson regression model including the four treatment arms separately. For these two regression models, initial individual MFD was associated with the appearance of new nodules. As shown in figures 16 and 17, these models reveal a strong interaction between the initial number of nodules and the predicted number of new nodules. It shows that in subjects treated 3-monthly (either with 150 or 800 µg/kg) fewer nodules had appeared than in subjects treated annually (with 150 or 800 µg/kg), and that the difference of appearance was highly correlated with the initial number of nodules harboured by the

participants. In addition, treatment with high IVM dose does not seem to influence the number of new nodules, regardless of the number of initial palpable nodules.

| | *Saturated model* | | *Stepwise model* | |
|---|---|---|---|---|
| **Appearance of nodule(s) (Yes/No)** | OR [95% CI] | *P* | OR [95% CI] | *P* |
| **Less than 5 nodules:** | | | | |
| 150 µg/kg 3-monthly (n=66) | Ref. | | Ref. | |
| 150 µg/kg annually (n=77) | 1.6 [0.6-3.8] | 0.320 | 1.6 [0.7-3.8] | 0.280 |
| 800 µg/kg 3-monthly (n=54) | 2.6 [0.8-7.7] | 0.092 | 2.6 [0.9-7.9] | 0.082 |
| 800 µg/kg annually (n=58) | 0.9 [0.4-2.1] | 0.777 | 0.9 [0.4-2.2] | 0.871 |
| **More than 5 nodules:** | | | | |
| 150 µg/kg 3-monthly (n=59) | 0.8 [0.3-1.9] | 0.619 | 0.9 [0.4-2.0] | 0.736 |
| 150 µg/kg annually (n=49) | 3.5 [0.9-13.3] | 0.066 | 4.1 [1.1-15.3] | 0.034 |
| 800 µg/kg 3-monthly (n=58) | 1.2 [0.4-2.9] | 0.759 | 1.3 [0.5-3.2] | 0.577 |
| 800 µg/kg annually (n=64) | 5.2 [1.4-19.5] | 0.014 | 5.5 [1.5-20.1] | 0.010 |
| **Age** | 1.0 [1.0-1.0] | 0.872 | | |
| **Weight** | 1.0 [1.0-1.0] | 0.661 | | |
| **CMFL in the village of residence** | | | | |
| Low | Ref. | | | |
| Middle | 1.2 [0.5-2.9] | 0.704 | | |
| High | 1.3 [0.6-3.0] | 0.537 | | |
| **MFD** | | | | |
| 0 – 30 mf/mg | Ref. | | | |
| 31 – 80 mf/mg | 1.3 [0.6-2.6] | 0.459 | | |
| 81 – 170 mf/mg | 1.2 [0.6-2.4] | 0.624 | | |
| More than 171 mf/mg | 1.7 [0.8-3.7] | 0.151 | | |

**Tableau 17. Logistic regression of the appearance of nodules**

OR: Odds-ratio; 95% CI: 95% confidence intervals; n: number of subjects; AIC: Akaike's Information Criterion; BIC: Bayesian Information Criterion; CMFL: community microfilarial load; MFD: microfilarial density

| | Saturated model | | Stepwise model | |
|---|---|---|---|---|
| | b [95% CI] | *P* | b [95% CI] | *P* |
| **Increase in no. of new nodules for each additional initial nodule*** | | | | |
| Annual treatment | 0.055 [0.030-0.080] | < 0.001 | 0.058 [0.035-0.081] | < 0.001 |
| 3-monthly treatment | 0.023 [0.0004-0.046] | 0.045 | 0.026 [0.005-0.048] | 0.014 |
| **Age** | 0.005 [0.0001-0.010] | 0.045 | 0.006 [0.002-0.009] | 0.002 |
| **Weight** | -0.007 [-0.005-0.003] | 0.703 | | |
| **CMFL in the village of residence** | | | | |
| Low | Ref. | | | |
| Middle | 0.089 [-0.153-0.332] | 0.470 | | |
| High | 0.149 [-0.074-0.372] | 0.191 | | |
| **MFD** | | | | |
| 0 – 30 mf/mg | Ref. | | Ref. | |
| 31 – 80 mf/mg | 0.084 [-0.108-0.277] | 0.389 | 0.102 [-0.081-0.285] | 0.275 |
| 81 – 170 mf/mg | 0.265 [0.082-0.447] | 0.004 | 0.290 [0.117-0.463] | 0.001 |
| More than 171 mf/mg | 0.358 [0.181-0.535] | < 0.001 | 0.383 [0.217-0.550] | <0.001 |
| *P* of the model | < 0.001 | | < 0.001 | |
| AIC | 1648 | | 1644 | |
| BIC | 1686 | | 1669 | |
| Log likelihood | - 815.2 | | -816.3 | |

**Tableau 18. Poisson regression model with pooled arms**

* The reference is defined as annual treatment and 2 initial nodules
b: regression coefficients; 95% CI: 95% confidence intervals

| | *Saturated model* | | *Stepwise model* | |
|---|---|---|---|---|
| | b [95% CI] | *P* | b [95% CI] | *P* |
| **Increase in no. of new nodules for each additional initial nodule*** | | | | |
| 150 µg/kg annually | 0.043 [0.014-0.071] | 0.004 | 0.046 [0.019-0.073] | 0.001 |
| 800 µg/kg annually | 0.067 [0.039-0.095] | <0.001 | 0.070 [0.043-0.096] | <0.001 |
| 150 µg/kg 3-monthly | 0.021 [-0.004-0.047] | 0.106 | 0.024 [-0.000-0.048] | 0.052 |
| 800 µg/kg 3-monthly | 0.026 [-0.001-0.053] | 0.063 | 0.029 [0.004-0.056] | 0.026 |
| **Age** | 0.005 [-0.00001-0.010] | 0.051 | 0.006 [0.002-0.009] | 0.003 |
| **Weight** | -0.001 [-0.005-0.003] | 0.659 | | |
| **CMFL in the village of residence** | | | | |
| Low | Ref. | | | |
| Middle | 0.090 [-0.154-0.333] | 0.470 | | |
| High | 0.151 [-0.072-0.375] | 0.185 | | |
| **MFD** | | | | |
| 0 – 30 mf/mg | Ref. | | Ref. | |
| 31 – 80 mf/mg | 0.089 [-0.103-0.281] | 0.364 | 0.105 [-0.079-0.288] | 0.263 |
| 81 – 170 mf/mg | 0.278 [0.095-0.461] | 0.003 | 0.301 [0.128-0.474] | 0.001 |
| More than 171 mf/mg | 0.371 [0.194-0.549] | < 0.001 | 0.394 [0.227-0.561] | < 0.001 |
| *P* of the *likelihood-ratio test* for the interaction term: 0.035 | | | | |
| *P* of the model | < 0.001 | | <0.001 | |
| AIC | 1649 | | 1645 | |
| BIC | 1695 | | 1678 | |
| *Log likelihood* | -813.5 | | -814.6 | |

**Tableau 19. Poisson regression model (with interaction term between initial number of nodules and treatment arms)**

* The reference is defined as 150 µg/kg annually and 2 initial nodules
b: regression coefficients; 95% CI: 95% confidence intervals

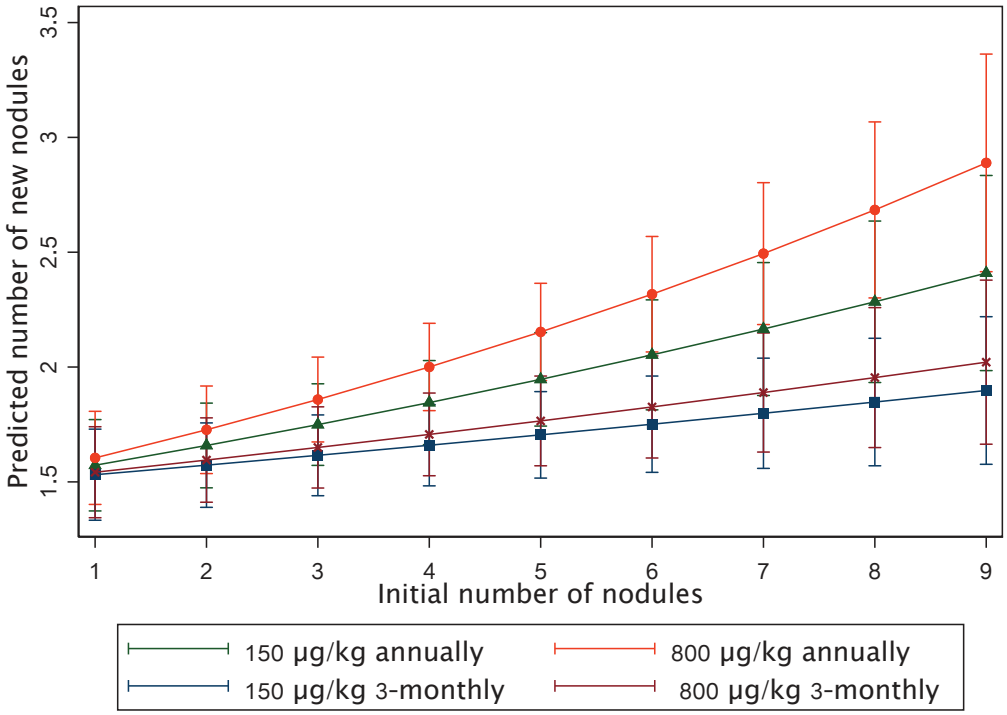


**Figure 16. Predicted number of new nodules vs initial number of nodules (four treatment arms)**

Bars indicate 96% confidence intervals.

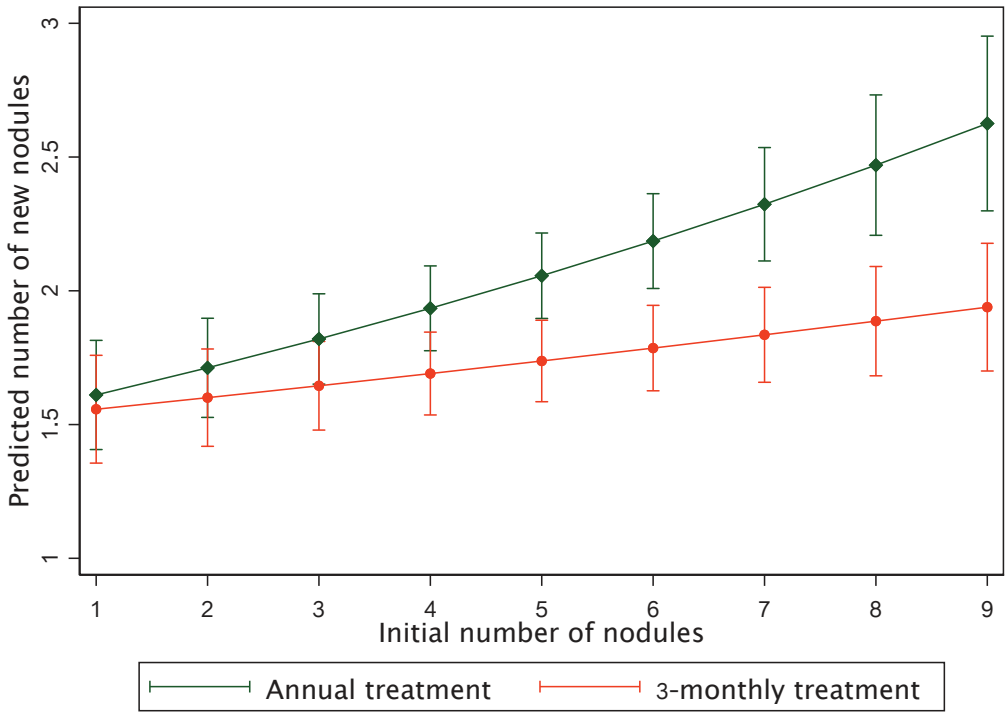


**Figure 17. Predicted number of new nodules vs initial number of nodules (annual vs 3-monthly**

Bars indicate 96% confidence intervals.

#### *4.3.4.3 Analysis of disappearance of nodules between 1994 and 1997*

In the 248 subjects treated annually, the mean number of nodules which disappeared between 1994 and 1997 (0.46) was lower than in the 237 subjects treated 3-monthly (0.60) but the difference was not significant (Student test: *P* = 0.0605). The mean numbers of nodules that disappeared between 1994 and 1997 in subjects of each treatment group are shown in Table 18. The ANOVA did not show any difference between the four groups (*P* = 0.279). In consequence, we did not perform further analysis on the disappearance of nodules.

### 4.3.5 Discussion

Our study is the first to compare the effect of various IVM treatment regimens on the appearance of new onchocercal nodules in human subjects exposed to transmission of *O. volvulus*. It demonstrates that the mean number of palpable nodules which appeared within the 3-year period of the trial was significantly lower in the individuals treated 3-monthly with IVM than in those treated annually, and that the high doses had no higher effect than standard doses in reducing the number of new nodules. Strangely, the difference between 800 µg/kg annually and 800 µg/kg 3-monthly was less marked than the difference between 150 µg/kg annually and 150 µg/kg 3-monthly, we have no biological clue to understand this difference, it may be due to unmeasured confounding factors (such as pharmacokinetics or pharmacodynamics characteristics) or to hazards due to serendipity.

We found no significant association between CMFL and appearance of nodules, we hypothesis that with a bigger effective, the association between CMFL exposition and appearance of new nodules may appear.

These results should be interpreted in the light of what is known on the biology of *O. volvulus* within the year following the infective bite. The modalities of development of *O. volvulus* from the initial penetration of the parasite into the host as an L3 to the time when it is found as an adult fecund stage in a nodule are not fully known. The time of the L3-L4 molt has been assessed by *in vitro* studies and by infecting experimentally various animals with L3s of *Onchocerca* sp. The time of the final molt was evaluated by following up the appearance of

antibodies, which were assumed to be stage-specific, in a simian model, and by an *in vitro* culturing system. It appears that, for *O. volvulus*, the L3-L4 molt starts 2-3 days after inoculation (Duke et al. 1991), that the final molt occurs between 1.5 and 2.5 months (Voronin et al. 2019), that the adults become sexually mature at 7.5-11 months, and that the first mf produced by the mature adult female worms can be detected after an average period of 12-15 months (Eberhard et al. 1991). Thus, the lifespan of the immature adult worms would range between 5 months (7.5 minus 2.5) and 9.5 months (11 minus 1.5). Given this timeframe, one can estimate that the proportion of L4s exposed to IVM in an individual treated 3-monthly will be between 50% (if the L4s' lifespan is 1.5 months) and 83% (if it is 2.5 months). Regarding immature worms, most would be exposed twice, and a small proportion three times, to the drug. Conversely, given their short lifespan, only a small proportion of L3s "inoculated" to these subjects treated every 90 days would be exposed to the drug. If one assumes that the appearance (or not) of new nodules reflects the effect of the drug on the parasitic stages preceding the mature adult stage, which is debatable (see below), the observed decrease of 17.7% in the number of new nodules in the subjects treated 3-monthly suggests that the prophylactic effect of IVM is not limited to the effects of the drug on the L3s or on the L3-L4 molting process only, but that IVM has also a partial effect on the L4s and/or the immature adult worms. IVM is known to kill L4s and young adults of the canine heartworm *Dirofilaria immitis* and to have a "slow kill" activity against *D. immitis* adults. Indeed, IVM is described as a very effective prophylactic drug against *D. immitis* infection in dogs. Furthermore, we know that the latter parasite is a very close relative to *Onchocerca spp*. Thus, we can hypothesis that IVM works in the same way in *Onchocerca volvulus* (McCall 2005).

In this study, we assumed that the effect of a drug on the L4s and immature adults can be assessed by following up the appearance of new nodules in hosts exposed to transmission of *O. volvulus*. This is debatable because the sites where the L4s and the immature adults live, and the modalities by which the adult female worms are finally found in a nodule, are poorly known. In particular, the extent to which immature females are attracted by existing nodules or are able to create a new nodule are not known. According to Duke *et al.*, "*it is expected … that the L4 will be highly mobile and capable (by means unknown) of locating the adult sites of election or (perhaps by means of pheromones) of finding pre-existing worm bundles; and that the immature females may continue these wanderings, …, but are then likely to settle down to form nodules of their own or to join pre-existing nodules. The possibility cannot be excluded that some of the immature*

*females may remain dormant at a prepubertal phase, situated in the connective tissues away from the nodules*" (Duke et al. 1991). Guderian *et al.* compared the sites of appearance of new nodules in a group of subjects from whom all the palpable nodules had been removed and a group with no nodulectomy. They concluded that "*It seems likely that young, female, unencapsulated O. volvulus are attracted to existing nodules, settle down next to them and then become encapsulated themselves"* (Guderian et al. 1997). However, as it is admitted (a) that the female worms, once in a nodule, stay there, and (b) that nodules form around female worms (not male worms), one may assume that the appearance of a new nodule can occur only if new females have appeared. Therefore, the lower number of new nodules recorded in the groups treated 3-monthly, when compared to the annually-treated group, results probably from an at least partial effect of ivermectin on the L4s and/or the juvenile female worms.

Specific study designs, using probably animal models, could be developed to evaluate the strength of this prophylactic effect after a single dose of IVM, which would help refine the mathematical models used to predict the impact of IVM MDA on onchocerciasis transmission intensity. Trials could also be conducted to define which treatment frequency would be required to obtain the best prophylactic effect. We found that 3-monthly treatments led to a significant reduction in the appearance of new nodules when compared to annual treatment but the difference was not very marked. Monthly treatments would probably lead to a stronger effect, as suggested by the results of studies conducted on the *O. ochengi*/cattle model (Tchakouté et al. 1999; Njongmeta et al. 2004). Such monthly treatments have been used in studies evaluating their possible macrofilaricidal effect on *O. volvulus* (Duke et al. 1990) or their effect on *Loa loa* (Kombila et al. 1998). They probably cannot be applied on a large scale, but could be proposed to individuals visiting temporarily an onchocerciasis endemic area. Unlike loiasis which can be prevented (totally) using diethylcarbamazine (DEC) (Duke 1963; T. B. Nutman et al. 1988), no drug is currently proposed to prevent onchocerciasis. Trials using DEC were conducted on chimpanzees experimentally infected with *O. volvulus* and on humans by looking at the effect of the drug on L3s, but the results were not conclusive (Duke 1968).

A remaining question is why the difference of impact between 3-monthly and annual treatment is higher when the initial number of nodules is higher. Before the start of this study, some participants had more nodules than others. This variability can be explained by different

levels of exposure to onchocerciasis transmission, but also by inter-individual heterogeneity in immunological response. Indeed, Tchakouté *et al.* observed the wide variation in susceptibility between cattle (even when controlling for blackfly exposure) (Tchakouté et al. 2006). Some individuals are more predisposed than others to tolerate incoming parasites, with a weaker immune response allowing more L3s' and L4s' developing to the adult stage, and therefore leading to more nodules. During the 3 years of the trial, it is very unlikely that these two factors changed for the participants. Thus, it makes sense that the most infected people (i.e. with the highest initial number of nodules and highest initial MFD) at the start of the study, are also the most infected ones at the end of the trial.

Few studies tried to evaluate the impact of IVM treatment on nodules' disappearance. Duke *et al*. assessed this phenomenon by comparing patients who were given IVM at 150 µg/kg 3-monthly and untreated persons. They described a higher proportion of nodules that had disappeared in the treated group but the difference was not significant (Duke et al. 1992). Three other studies reported that nodules can disappear after repeated doses of IVM (Emukah et al. 2004; Ukaga et al. 2001; Anosike et al. 2012) . We did not find a difference in the number of nodules that disappeared between the treatment arms of our study, but this could be due to a lack of statistical power due to a small sample size. Further studies have to be conducted to determine the impact of repeated doses of IVM on the nodules' disappearance.

### 4.3.6 Conclusions

**Cette étude apporte la preuve que les traitements trimestriels sont plus efficaces que les traitements annuels pour prévenir l'apparition de nodules onchocerquiens. Cet effet est particulièrement marqué chez les individus présentant un grand nombre de nodules avant le traitement. Nos résultats soutiennent, pour la première fois, que l'ivermectine a probablement un effet prophylactique sur les stades L4s et/ou les femelles juvéniles d'*O. volvulus.* Lorsque le médicament est administré à 3 mois d'intervalle, cet effet n'est que partiel, mais il pourrait être plus efficace lorsqu'il est administré à des intervalles plus courts.**

# 4.4 Étude de pharmacovigilance systématique : effets indésirables graves associés à l'ivermectine

## Serious adverse reactions associated with ivermectin: a systematic pharmacovigilance study in sub-Saharan Africa and in the rest of the World

*PLOS Neglected Tropical Diseases*

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### 4.4.1 Abstract

**Background**. Ivermectin is known to cause severe encephalopathies in subjects infected with loiasis, an endemic parasite in Sub-Saharan Africa (SSA). In addition, case reports have described ivermectin-related serious adverse drug reactions (sADRs) such as toxidermias, hepatic and renal disorders. The aim of this study was to identify suspected sADRs reported after ivermectin administration in VigiBase, the World Health Organization's global individual case safety reports database and analyze their frequency relative to the frequency of these events after other antinematodal drugs reported in SSA and other areas of the world (ROW).

**Methods**. All antinematodal-related sADRs were extracted from VigiBase. Disproportionality analyses were conducted to investigate nervous, cutaneous, psychiatric, respiratory, renal, hepatic and cardiac suspected sADRs reported after ivermectin and benzimidazole drug administration across the world, in SSA and RoW.

**Principal findings**. 2041 post-ivermectin or post-benzimidazole suspected sADRs were identified including 667 after ivermectin exposure (208 in SSA and 459 in the RoW). We found an increased reporting for toxidermias, encephalopathies, confusional disorders after ivermectin compared to benzimidazole drug administration. Encephalopathies were not only reported from SSA but also from the RoW (adjusted reporting odds ratios [aROR] 6.30, 95% confidence interval: 2.68–14.8), highlighting the fact these types of sADR occur outside loiasis endemic regions.

**Conclusion.** We described for the first time suspected sADRs associated with ivermectin exposure according to geographical origin. While our results do not put in question ivermectin's excellent safety profile, they show that as for all drugs, appropriate pharmacovigilance for adverse reactions is indicated.

**Author summary.**

Ivermectin is a drug used worldwide for various indications: onchocerciasis, lymphatic filariasis, strongyloidiasis, human sarcoptic scabies, acarodermatitis and rosacea.

In the early 1990s, it was discovered that ivermectin could induce severe encephalopathies in some patients with high parasite loads of *Loa loa*, a filarial nematode. This objective of this pharmacovigilance study is to summarize serious neurological and non-neurological post-ivermectin adverse drug reactions reported in the World Health Organization

database called VigiBase. This study shows that reported serious adverse drug reactions associated with ivermectin are fairly consistent with those mentioned in the official product information of ivermectin but also provides some new signals. Serious post-ivermectin encephalopathies can also occur outside of *Loa loa* endemic regions but the understanding of the mechanism by which it occurs requires further studies. A new signal concerning two serious toxidermias (DRESS syndrome and acute generalized exanthematous pustulosis) is also described. A lack of reporting of adverse drug reactions is noticeable in some Sub-Saharan African countries, and actions are needed to increase the reporting rates of these adverse effects in these countries.

### 4.4.2 Introduction

Ivermectin is included in the World Health Organization (WHO) list of essential medicines and is commonly used worldwide. Stromectol (ivermectin 3 mg) and its generics (Arrow Lab, Biogaran, Gerda, Mylan, Pierre Fabre, Sandoz, Zentiva) are mainly distributed in Europe and North America. In Europe, ivermectin is labeled for the treatment of strongyloidiasis, diagnosed or suspected infection with *Wuchereria bancrofti* (the filarial nematode causing lymphatic filariasis) or *O. volvulus* (the filarial nematode causing onchocerciasis), and human sarcoptic scabies. In North America, ivermectin is labeled for the treatment of strongyloidiasis and onchocerciasis. Ivermectin is also used off-label in certain cases of acarodermatitis (skin inflammation due to bites of parasitic mites), rosacea and loiasis (the disease caused by the filarial nematode *Loa loa*) (ANSM n.d.; FDA n.d.). In African countries, ivermectin is distributed at single oral doses of 150-200 µg/kg as part of onchocerciasis and lymphatic filariasis elimination programs (the drug, registered under the name of Mectizan for these indications, is donated by Merck & Co., Inc.). It is used as preventive chemotherapy, i.e. distributed annually (sometimes biannually) using a mass drug administration strategy, i.e. to the entire eligible population of the target communities without individual diagnosis.

Ivermectin is a derivative of avermectins. It acts mainly by binding to the glutamate-dependent chloride channels of invertebrate nerve and muscle cells, causing an increase in membrane permeability leading ultimately to neuromuscular paralysis and death of certain parasites. In subjects with high densities of microfilariae (mf, the larval stages of the filarial

parasites) in the skin or the blood, ivermectin is able to induce complex inflammatory reactions called Mazzotti reactions which include pruritus, rash, fever, malaise, lymphadenopathy, arthralgia, tachycardia, hypotension, edema and abdominal pain (Duke 1990; Ackerman et al. 1990). These reactions reflect the inflammatory phenomena associated with the destruction of mf by the drug. Since the early 1990, ivermectin has been known to cause potentially fatal encephalopathies in individuals with very high microfilarial density of *L. loa* in the blood (loiasis is endemic only in Central Africa) (Boussinesq et al. 2003; Gardon, Gardon-Wendel, Demanga, et al. 1997), also referred to as "Possible/Probable *L. loa* encephalopathy temporally related to Mectizan" (PLERM). PLERM can occur in subjects with *L. loa* microfilarial density >10,000 mf/mL if measured before treatment or >1,000 mf/mL if measured after treatment (Twum-Danso and Meredith 2003). Since then, few studies have been conducted to investigate the frequency of these *Loa*-related adverse drug reactions (ADR) and the mechanisms by which they occur (Chesnais et al. 2020) .

In 2017, an analysis of the WHO Global individual case safety report (ICSR) database (VigiBase) for serious neurological adverse events was conducted (Nzolo et al. 2017). The search identified 52 ivermectin-related ICSRs entered into VigiBase by the pharmacovigilance system of the Democratic Republic of the Congo (DRC) between 2009 and 2013. All patients had central and peripheral nervous system disorders. The mean *L. loa* microfilarial density measured after treatment in these patients was 2149.1 mf/mL, and 61% of them had microfilarial density below 1000 mf/mL, suggesting the possible occurrence of PLERM at low microfilarial density. Another search of the VigiBase was conducted in 2016 to identify serious neurological adverse events other than PLERM after ivermectin administration. The authors found 28 cases of suspected neurological serious ADRs (sADRs) following ivermectin treatment for diseases other than onchocerciasis (10 for scabies, 8 for acarodermatitis, 3 for strongyloidiasis, 5 for lymphatic filariasis, 1 for myiasis and 1 for taeniasis) (Chandler 2018). This study raised questions about the mechanisms underlying the appearance of these neurological effects. To our knowledge, these studies are the only two that have used a pharmacovigilance database to evaluate the occurrence of post-ivermectin ADRs and they have focused exclusively on neurological events.

Our systematic search of the literature for non-neurological adverse events found 10 cases where ivermectin was associated with cutaneous reactions (Aroke et al. 2017; Ngwasiri et al. 2018; Mara et al. 2004; Sanz-Navarro et al. 2017; Fujimoti et al. 2014), nephropathy (Cruel et al. 1997), psychiatric disorders (Kaur et al. 2017; Mohapatra and Sahoo 2015), hepatic disorders

(Sparsa et al. 2006; Veit et al. 2006) and multiorgan dysfunction syndrome (Choksi et al. 2016). Clinical trials and observational studies have reported common adverse events such as headache, pruritus, muscle pain, cough, dyspnea, nausea, vomiting, diarrhea, blurred vision, postural hypotension and confusion and more anecdotal effects such as serious skin reactions and edematous swelling (Budge et al. 2018; De Sole et al. 1989; Burnham 1993).

In the present study, we searched VigiBase for all the suspected sADRs (not only the neurological ones) reported after ivermectin treatment and after treatment with other antinematodal drugs and conducted disproportionality analyses considering the geographical origin of the reported cases. More specifically, the aims of this study were to identify (i) possible non-neurological pharmacovigilance signals (increased reporting of serious suspected adverse reactions after treatment with ivermectin compared to treatment with other antinematodal drugs), and (ii) possible neurological signals related to indications other than onchocerciasis.

## 4.4.3 Methodology

### *4.4.3.1 Data source*

Data were extracted from the WHO Global Individual Case Safety Report (ICSR) database, VigiBase [25] which includes more than 20 million cases of suspected ADRs reported by national pharmacovigilance centers in more than 130 countries participating in the WHO Program for International Drug Monitoring [26]. An ICSR is an anonymized report for a single individual who experienced adverse event(s) that may be linked to the use of one or more drugs. ICSR contains sociodemographic information (age, sex, reporter qualification, country of origin, year of report), information about the drug administration (frequency, dosage, co-medication) and information about the reported adverse event. The latter include the seriousness according to the criteria of the International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use (ICH) [27], adverse event verbatim description and associated terms from the Medical Dictionary for Regulatory Activities (MedDRA) developed by the ICH. All reports of suspected sADRs associated with antinematodal drugs (Anatomical Therapeutic Chemical [ATC] code P02C) from December 2003 (first ever report of ivermectin-associated suspected sADR recorded) up to July 15, 2020 were extracted. Antinematodal drugs included ivermectin,

benzimidazole drugs (mebendazole, tiabendazole, albendazole, ciclobendazole, flubendazole, fenbendazole), levamisole, pyrantel, piperazine, diethylcarbamazine, and pyrvinium. Prior to analysis, suspected duplicate reports identified by an automated screening were excluded [28]. When ivermectin had been administered in combination with a benzimidazole or another antinematodal drug, the report was excluded from the analysis. Suspected sADRs were classified following the MedDRA [29], grouped at the System Organ Class (SOC) level and at the individual preferred term (PT) level.

#### *4.4.3.2 Study design*

We performed disproportionality analyses using the case/non-case method which allows to identify disproportionate reporting, *i.e.* a higher than expected number of adverse reaction reports compared to other reactions recorded in the database by calculating Reporting Odds Ratios (ROR). ROR compares the odds of exposure to ivermectin between cases and non-cases (Moore et al. 2005; Puijenbroek et al. 2002).

Cases were defined as reports of each suspected sADR of interest identified by a MedDRA PT for severe headache, encephalopathies, confusional disorders, seizures, toxidermias (drug reaction with eosinophilia and systemic symptoms, Stevens-Johnson syndrome, toxic epidermal necrolysis and acute generalized exanthematous pustulosis), psychiatric disorders, suicidal behavior, severe acute respiratory syndrome (SARS), renal disorders, hepatic disorders, cardiac failure, rhythm disorders and Mazzotti reaction. For specific syndromes of interest, we mapped the PTs for the most common symptoms to one variable and used that in the analyses instead of the individual PTs (Table 20).

Non-cases were defined as reports of any other suspected sADR occurring after administration of the same drug.

#### *4.4.3.3 Exposure definition*

Exposure to ivermectin was identified in the ICSR by the use of ivermectin (ATC code P02CF01) preceding the onset of the serious adverse reaction. Only oral administration of ivermectin was included (topical formulations were excluded).

#### *4.4.3.4 Statistical analysis*

Descriptive statistics were used to summarize the basic characteristics according to the origin of the ICSR: sub-Saharan Africa (SSA) or the rest of the world (RoW).

Among all suspected sADR reports associated with antinematodal drugs, our primary analyses consisted in calculating the ROR of each suspected sADR of interest (and corresponding 95% confidence interval [95% CI]) for ivermectin compared to benzimidazole drugs using logistic regression models adjusted for age groups, date of the ICSR publication, and origin of the notification (SSA or RoW). The latter can additionally be used as a proxy for ivermectin indication since >99% of subjects with onchocerciasis live in SSA, and ivermectin is usually not used for lymphatic filariasis control/elimination outside SSA. To explore a potential effect modification by origin, we performed secondary analyses with stratification according to the origin of the notification (SSA and RoW).

Sensitivity analyses were performed using all antinematodal drugs (including benzimidazoles) as the control group instead of benzimidazoles alone and using the same statistical methods.

For all analyses, the p-values in the Tables are indicated by asterisks: ***: $p<0.01$; **: $p\geq0.01$ to $<0.05$; *: $p\geq0.05$ to $<0.10$. For all analyses, "N/A" means that the category is not available or non-applicable.

Analyses were conducted using STATA v.15.1 software (StatCorps, LP, College Station, TX, USA). Maps were created using the mapCountryData package from R statistical software v. 3.5.0.

| New variable | Algorithm |
|---|---|
| **Encephalopathies** | **At least one of the following PTs**<br>– Confusion<br>– Aphasia<br>– Loss of consciousness<br>– Coma |
| **Confusional disorders** | **At least one of the following PTs**<br>– Confusion<br>– Agitation<br>– Disorientation |
| **Toxidermias** | **At least one of the following PTs**<br>– Drug reaction with eosinophilia and systemic symptoms syndrome<br>– Stevens-Johnson syndrome<br>– Toxic epidermal necrolysis<br>– Acute generalized exanthematous pustulosis |
| **Psychiatric disorders** | **At least one of the following PTs**<br>– Delusion<br>– Hallucination<br>– Delirium<br>– Depersonalization<br>– Derealization |
| **Renal disorders** | **At least one of the following PTs**<br>– Renal failure<br>– Renal impairment<br>– Renal pain<br>– Renal injury |
| **Hepatic disorders** | **At least one of the following PTs**<br>– Hepatitis<br>– Hepatic failure<br>– Hepatocellular injury<br>– Jaundice<br>– Liver injury<br>– Hepatic function abnormal |
| **Mazzotti reaction** | **At least two of the following PTs**<br>– Headache<br>– Asthenia **or** Fatigue<br>– Pyrexia **or** Chills<br>– Arthralgia **or** Myalgia<br>– Edema **or** Swelling |

**Tableau 20. Mapping of the PTs for the most common symptoms of syndrome of interest to a new variable**

## 4.4.4 Results

### *4.4.4.1 Descriptive analysis of the sADRs reported after ivermectin*

After elimination of duplicates, 2041 suspected sADRs occurring after administration of antinematodal agents were reported between December 2003 and July 2020, of which 209 (10.2%) resulted in death. A total of 667 suspected sADRs were reported after ivermectin administration: 208 cases in SSA and 459 in the RoW. Table 21 shows the distribution of cases between SSA and RoW by age, gender, who reported the case, brand name, fatality, reporting period, and indication.

Most cases concerned people aged 18-44 years old (43.7%) and were reported by healthcare professionals (90.0%). Mean age (44.7 ± 22.9 years for all cases) was significantly lower for SSA (32.3 ± 14.6 years) than for RoW cases (51.1 ± 23.8 years). Sex distribution was also significantly different between SSA cases (female:male ratio 1:1.96) and RoW cases (1:1). Stromectol, the most frequently brand name reported in the RoW (62.0%), was not reported at all in SSA. Suspected sADRs were more frequently fatal in the RoW (67 deaths; 14.6%) than in SSA (9 deaths; 4.3%). Onchocerciasis was the most frequently reported indication for ivermectin use and this was particularly the case in SSA. Scabies was the second most frequently reported indication for ivermectin use, all cases being from the RoW (96; 28.0%). The reported SOC are presented in Table 22, the three most reported SOC were “General disorders and administration site conditions” (44.4%), “Nervous system disorders” (31.3%) and “Skin and subcutaneous tissue disorders” (30.4%).

The three countries that reported the highest number of cases were the United States of America (152 ICRS, 22.8%), France (151, 22.6%) and the DRC (115, 17.2%). Distributions by country for the 6 most frequently reported SOC (excluding the SOC “Infections and Infestations” and “Injury, poisoning and procedural complications” for which a causal relationship to drug administration is extremely unlikely) are presented across the world in Figure 18 and across Africa and Europe in Figures 19 and 20, respectively.

The most frequently reported suspected sADRs are presented by SOC in Tables 23, 24 and 25. The ten most frequently reported suspected sADRs of interest are reported in Table 26.

| Characteristics | Sub-Saharan cases (n=208) | RoW cases (n=459) | Total (n=667) |
|---|---|---|---|
| **Age**, n (%) | | | |
| 0-17 | 23 (11.5%) | 32 (8.3%) | 55 (9.4%) |
| 18-44 | 136 (68.3%) | 119 (31.0%) | 255 (43.7%) |
| 45-64 | 36 (18.0%) | 110 (28.6%) | 146 (25.0%) |
| 65-74 | 3 (1.5%) | 45 (11.7%) | 48 (8.2%) |
| >74 | 1 (0.5%) | 78 (20.3%) | 79 (13.5%) |
| Unknown | 9 | 75 | 84 |
| **Gender**, n (%) | | | |
| Male | 137 (66.2%) | 221 (50.0%) | 358 (55.2%) |
| Female | 70 (33.8%) | 221 (50.0%) | 291 (44.8%) |
| Unknown | 1 | 17 | 18 |
| **Reporter type**, n (%) | | | |
| Healthcare professionals | 185 (94.9%) | 380 (87.8%) | 565 (90.0%) |
| Non-healthcare professionals | 10 (5.1%) | 53 (12.2%) | 63 (10.0%) |
| Unknown | 13 | 26 | 39 |
| **Brand name, n (%)** | | | |
| Stromectol | 0 | 285 (62.0%) | 285 (42.7%) |
| Mectizan | 100 (48.1%) | 8 (1.7%) | 108 (16.2%) |
| Others* | 0 | 45 (9.8%) | 45 (6.7%) |
| Unknown | 108 (51.9%) | 122 (26.6%) | 229 (34.3%) |
| **Fatal**, n (%) | | | |
| Yes | 9 (4.3%) | 67 (14.6%) | 76 (11.4%) |
| No | 199 (95.7%) | 392 (85.4%) | 591 (88.6%) |
| **Reporting period**, n (%) | | | |
| ≤ 2012 | 91 (43.7%) | 86 (18.7%) | 177 (26.5%) |
| 2013 - 2015 | 33 (15.9%) | 144 (31.4%) | 177 (26.5%) |
| 2016 - 2018 | 70 (33.6%) | 142 (30.9%) | 212 (31.8%) |
| 2019 - 2020 | 14 (6.7%) | 87 (18.9%) | 101 (15.1%) |
| **Indications**, n (%) | | | |
| Onchocerciasis | 110 (74.8%) | 10 (2.9%) | 120 (24.5%) |
| Scabies | 0 | 96 (28.0%) | 96 (19.8%) |
| Acarodermatitis | 0 | 80 (23.3%) | 80 (16.3%) |
| Strongyloidiasis | 1 (0.7%) | 64 (18.6%) | 65 (13.3%) |
| Filariasis | 29 (19.7%) | 7 (2.0%) | 36 (7.3%) |
| Rosacea | 0 | 27 (7.9%) | 27 (5.5%) |
| Parasitosis | 1 (0.7%) | 20 (5.8%) | 21 (4.3%) |
| Others** | 6 (4.0%) | 39 (11.4%) | 45 (9.2%) |
| Unknown | 61 | 116 | 177 |

**Tableau 21. Characteristics of sADRs exposed to ivermectin reported in VigiBase according to geographical origin**

* Soolantra (28), Scabioral (7), Sklice (6), Rosiver (2), Driponin (1), Ivermec (1).

** Error (10), Lice (10), Prophylaxis (6), Skin disease (4), Pruritus (3), Cysticercosis (3), Helminth infection (2), Hookworm (2), Schistosomiasis (2), Loiasis (1), Taenia (1), Worms (1).

| **System Organ Class (SOC)**, n (% of ICSR with mention of the SOC) | **Sub-Saharan reports** | **RoW reports** | **Total reports** |
|---|---|---|---|
| General disorders and administration site conditions | 120 (57.7%) | 176 (38.3%) | 296 (44.4%) |
| Nervous system disorders | 112 (53.8%) | 97 (21.1%) | 209 (31.3%) |
| Skin and subcutaneous tissue disorders | 72 (34.6%) | 131 (28.5%) | 203 (30.4%) |
| Gastrointestinal disorders | 51 (24.5%) | 78 (17.0%) | 129 (19.3%) |
| Infections and infestations | 5 (2.4%) | 77 (16.8%) | 82 (12.3%) |
| Musculoskeletal, connectives tissues disorders | 52 (25.0%) | 26 (5.7%) | 78 (11.7%) |
| Injury, poisoning, procedural complications | 0 | 67 (14.8%) | 67 (10.0%) |
| Psychiatric disorders | 20 (9.6%) | 42 (9.2%) | 62 (9.3%) |
| Respiratory, thoracic, mediastinal disorders | 10 (4.8%) | 52 (11.3%) | 62 (9.3%) |
| Renal and urinal disorders | 28 (13.5%) | 29 (6.3%) | 57 (8.5%) |
| Investigations | 1 (0.5%) | 56 (12.2%) | 57 (8.5%) |
| Eye disorders | 28 (13.5%) | 21 (4.6%) | 49 (7.3%) |
| Hepatobiliary disorders | 1 (0.5%) | 47 (10.2%) | 48 (7.2%) |
| Vascular disorders | 23 (11.1%) | 24 (5.2%) | 47 (7.0%) |
| Blood and lymphatic system disorders | 2 (1.0%) | 42 (9.2%) | 44 (6.6%) |
| Cardiac disorders | 1 (0.5%) | 27 (5.9%) | 28 (4.2%) |
| Metabolism and nutrition disorders | 0 | 24 (5.2%) | 24 (3.6%) |
| Immune system disorders | 2 (1.0%) | 13 (2.8%) | 15 (2.2%) |
| Ear and labyrinth disorders | 6 (2.9%) | 8 (1.7%) | 14 (2.1%) |
| Reproductive system and breast disorders | 6 (2.9%) | 3 (0.7%) | 9 (1.3%) |
| Pregnancy, puerperium, perinatal disorders | 0 | 9 (2.0%) | 9 (1.3%) |
| Neoplasm benign, malignant and unspecified | 0 | 8 (1.7%) | 8 (1.2%) |
| Endocrine disorders | 0 | 7 (1.5%) | 7 (1.0%) |
| Social circumstances | 0 | 4 (0.9%) | 4 (0.6%) |
| Surgical and medical procedures | 0 | 4 (0.9%) | 4 (0.6%) |
| Product issues | 0 | 3 (0.7%) | 3 (0.4%) |
| Congenital, familial and genetic disorders | 0 | 1 (0.2%) | 1 (0.1%) |

**Tableau 22. Frequency of reported SOC by regions and in total.**

Multiple SOC can be reported in a single ICSR

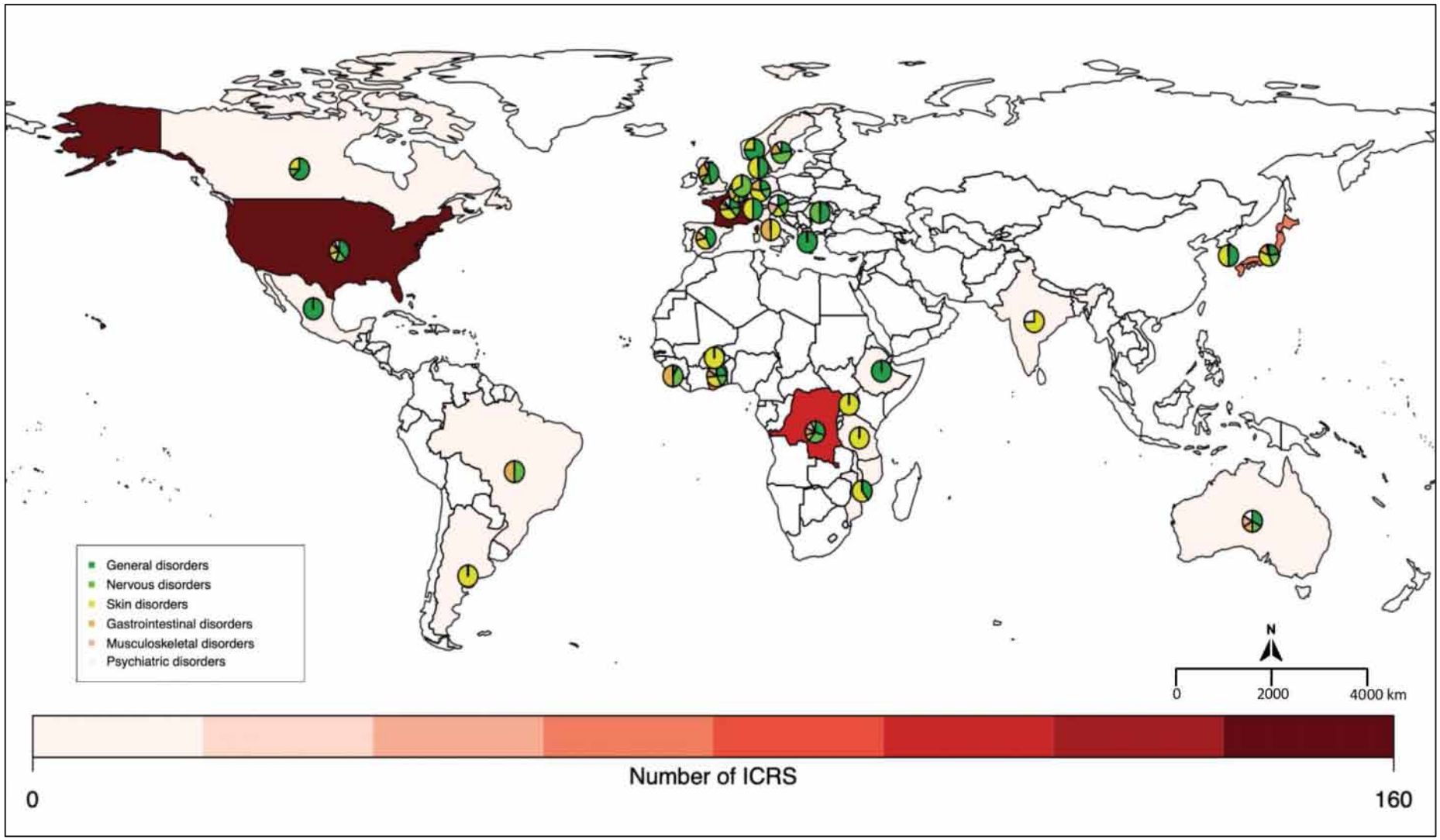


**Figure 18. Number of Individual Case Safety Reports (ICSRs) per country and distribution of sADRs of the 6 most reported SOCs**

The pie charts show the proportion of the most reported SOCs described by country. The number next to the pie chart represents the number of ICSRs by country (an ICSR can contain multiple SOCs).

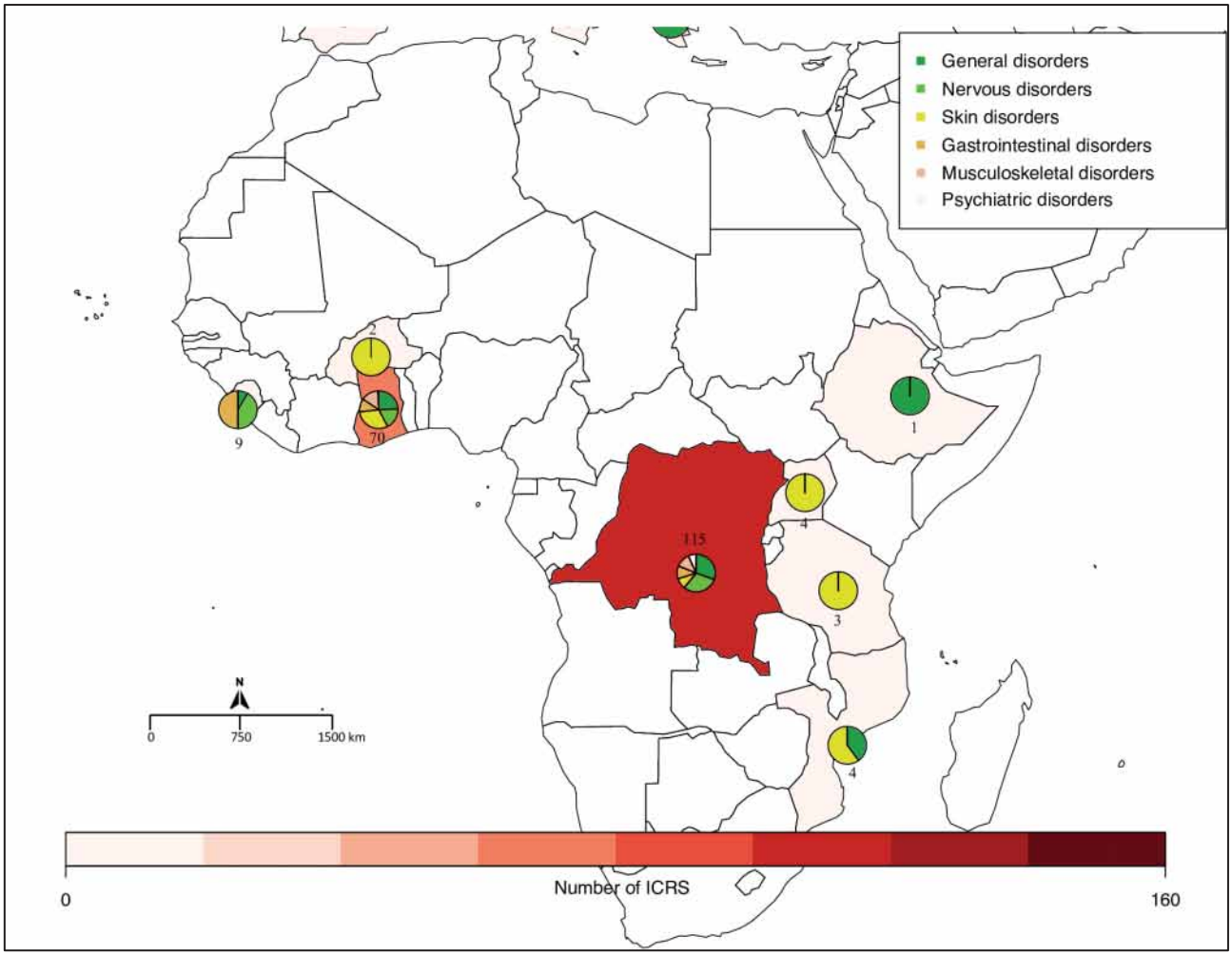


**Figure 19. Number of Individual Case Safety Reports (ICSRs) per African country and distribution of serious ADRs of the 6 most reported SOCs**

The pie charts show the proportion of the most reported SOCs described by country. The number next to the pie chart represents the number of ICSRs by country (an ICSR can contain multiple SOCs).

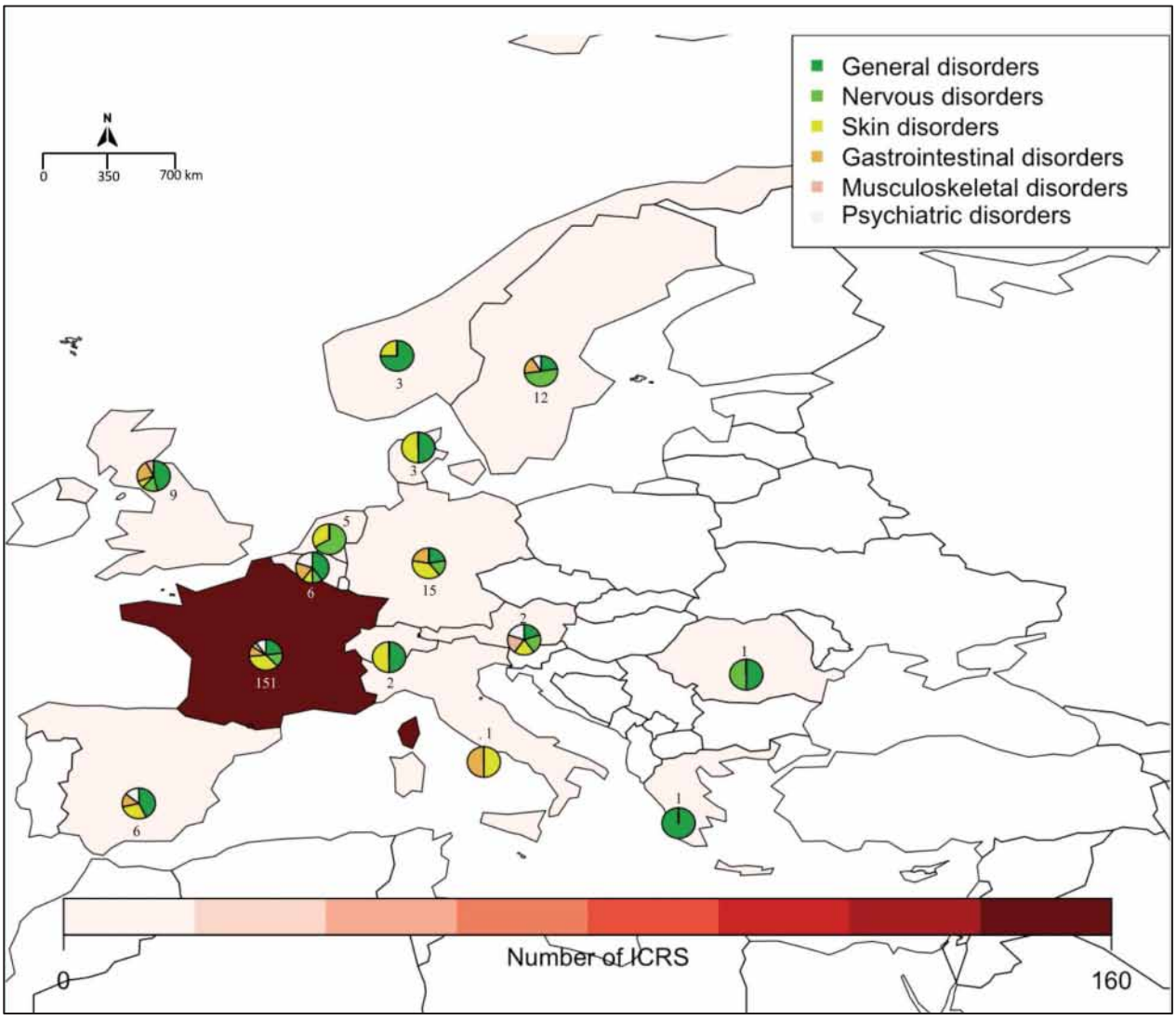


**Figure 20. Number of Individual Case Safety Reports (ICSRs) per European country and distribution of sADRs of the 6 most reported SOCs**

The pie charts show the proportion of the most reported SOCs described by country. The number next to the pie chart represents the number of ICSRs by country (an ICSR can contain multiple SOCs).

| SOC (number of serious ADR reports) | Most frequently reported serious ADR | | | | |
|---|---|---|---|---|---|
| | 1st reported (n) | 2nd reported (n) | 3rd reported (n) | 4th reported (n) | 5th reported (n) |
| **General disorders and administration site conditions (296)** | Asthenia (78) | Pyrexia (63) | Drug ineffective (38) | Gait disturbance (28) | Pain (19) |
| **Nervous system disorders (209)** | Headache (82) | Dizziness (43) | Coma (36) | Depressed level of consciousness (20) | Loss of consciousness (16) |
| **Skin and subcutaneous tissue disorders (203)** | Pruritus (76) | Rash (31) | Erythema (15) | Stevens-Johnson syndrome (15) | Rash maculo-papular (14) |
| **Gastrointestinal disorders (129)** | Vomiting (34) | Diarrhea (30) | Abdominal pain (23) | Nausea (12) | Dysphagia (9) |
| **Infections and infestations (82)** | Strongyloidiasis (34) | Pneumonia (11) | Sepsis (7) | Conjunctivitis (5) | Acarodermatitis (5) |
| **Musculoskeletal, connectives tissues disorders (78)** | Back pain (28) | Arthralgia (27) | Myalgia (20) | Pain in extremity (10) | Musculoskeletal pain (4) |
| **Injury, poisoning, procedural complications (67)** | Off label use (15) | Accidental exposure (10) | Incorrect route of administration (6) | Exposure during pregnancy (4) | Product use in unapproved indication (4) |
| **Psychiatric disorders (62)** | Confusional state (13) | Abnormal behavior (11) | Agitation (9) | Disorientation (5) | Hallucination (4) |
| **Respiratory, thoracic, mediastinal disorders (62)** | Dyspnea (18) | ARDS (7) | Cough (7) | Respiratory failure (5) | Asthma (4) |
| **Renal and urinal disorders (57)** | Urinary incontinence (21) | Acute kidney injury (8) | Chromaturia (4) | Oliguria (4) | Hematuria (3) |
| **Investigations (57)** | Weight decreased (5) | Hepatic enzyme increased (5) | Platelet count decreased (4) | CRP increased (3) | Heart rate increased (3) |
| **Eye disorders (49)** | Conjunctival haemorrhage (21) | Eye pain (5) | Vision blurred (4) | Visual impairment (4) | Blindness (3) |
| **Hepatobiliary disorders (48)** | Hepatitis (10) | Hepatocellular injury (9) | Jaundice (5) | Liver disorder (5) | Hepatic failure (4) |
| **Vascular disorders (47)** | Hypotension (20) | Hypertension (7) | Hematoma (3) | Circulatory collapse (2) | Orthostatic hypotension (2) |
| **Blood and lymphatic system disorders (44)** | Eosinophilia (18) | Lymphadenopathy (5) | Agranulocytosis (4) | Anemia (4) | Thrombocytopenia (4) |
| **Cardiac disorders (28)** | Cardiac arrest (5) | Palpitations (5) | Tachycardia (5) | Cardiac failure (4) | Cardio-respiratory arrest (3) |
| **Metabolism and nutrition disorders (24)** | Decreased appetite (5) | Hyperkaliemia (4) | Dehydration (3) | Hyponatremia (3) | Hyperglycemia (2) |
| **Immune system disorders (15)** | Hypersensitivity (7) | Anaphylactic shock (4) | Anaphylactic reaction (1) | Drug hypersensitivity (1) | Immune system disorder (1) |
| **Ear and labyrinth disorders (14)** | Vertigo (9) | Ear pain (2) | Tinnitus (2) | Deafness (1) | Ototoxicity (1) |
| **Reproductive system and breast disorders (9)** | Pelvic pain (2) | Scrotal oedema (2) | Menstruation irregular (1) | Oedema genital (1) | Penile swelling (1) |
| **Pregnancy, puerperium, perinatal disorders (9)** | Abortion spontaneous (4) | Foetal death (2) | Oligohydramnios (1) | Premature delivery (1) | Stillbirth (1) |
| **Neoplasm benign, malignant, unspecified (8)** | Hodgkin's disease (3) | Hydatidiform mole (1) | Large B-cell lymphoma (1) | Glioblastoma (1) | T-cell lymphoma (1) |
| **Endocrine disorders (7)** | Hypothyroidism (2) | Adrenal insufficiency (1) | Diabetes insipidus (1) | Adrenocortical insufficiency (1) | Autoimmune thyroiditis (1) |
| **Social circumstances (4)** | Loss of independence (2) | Immobile (1) | Impaired driving ability (1) | | |
| **Surgical and medical procedures (4)** | Central catheterization (1) | Limb operation (1) | Spinal operation (1) | Mechanical ventilation (1) | Endotracheal intubation (1) |
| **Product issues (3)** | Product taste abnormal (1) | Wrong label (1) | Product substitution issue (1) | Product availability issue (1) | |
| **Congenital, familial and genetic disorders (1)** | Congenital anomaly (1) | | | | |

**Tableau 23. Most frequently reported serious ADR associated with ivermectin for each System Organ Class (SOC)**

If several sADRs belonging to the same SOC are reported in a single patient (ICSR form), the SOC is counted only once in the total.

| SOC (number of serious ADR reports) | Most frequently reported serious ADR | | | | |
|---|---|---|---|---|---|
| | 1st reported (n) | 2nd reported (n) | 3rd reported (n) | 4th reported (n) | 5th reported (n) |
| **General disorders and administration site conditions (120)** | Asthenia (61) | Pyrexia (42) | Gait disturbance (22) | Pain (15) | Chills (9) |
| **Nervous system disorders (112)** | Headache (60) | Coma (31) | Dizziness (29) | Depressed level of consciousness (13) | Loss of consciousness (7) |
| **Skin and subcutaneous tissue disorders (72)** | Pruritus (47) | Rash (9) | Steven-Johnson syndrome (8) | Rash pruritic (4) | Angioedema (2) |
| **Musculoskeletal, connectives tissues disorders (52)** | Back pain (22) | Arthralgia (20) | Myalgia (14) | Musculoskeletal pain (3) | Neck pain (3) |
| **Gastrointestinal disorders (51)** | Diarrhea (21) | Vomiting (17) | Abdominal pain (9) | Anal incontinence (5) | Abdominal distension (2) |
| **Eye disorders (28)** | Conjunctival haemorrhage (19) | Eye pain (5) | Corneal opacity (1) | Eye discharge (1) | Eye swelling (1) |
| **Renal and urinal disorders (28)** | Urinary incontinence (19) | Oliguria (3) | Chromaturia (2) | Bladder sphincter atony (2) | Dysuria (1) |
| **Vascular disorders (23)** | Hypotension (16) | Hypertension (5) | Hematoma (1) | Blood pressure inadequately controlled (1) | |
| **Psychiatric disorders (20)** | Abnormal behavior (9) | Agitation (6) | Disorientation (3) | Apathy (2) | Insomnia (1) |
| **Respiratory, thoracic and mediastinal disorders (10)** | Cough (4) | Catarrh (3) | Oropharyngeal pain (2) | Respiratory disorder (1) | |
| **Reproductive system and breast disorders (6)** | Pelvic pain (2) | Oedema genital (1) | Scrotal oedema (1) | Testicular pain (1) | Testicular swelling (1) |
| **Ear and labyrinth disorders (6)** | Vertigo (4) | Ear pain (2) | | | |
| **Infections and infestations (5)** | Cellulitis (1) | Conjunctivitis (1) | Pyuria (1) | Sepsis (1) | Necrotizing soft tissue infection (1) |
| **Blood and lymphatic system disorders (2)** | Jaundice acholuric (1) | Lymphadenitis (1) | | | |
| **Immune system disorders (2)** | Anaphylactic shock (1) | Hypersensitivity (1) | | | |
| **Cardiac disorders (1)** | Palpitations (1) | | | | |
| **Hepatobiliary disorders (1)** | Jaundice (1) | | | | |
| **Investigations (1)** | Pulse abnormal (1) | | | | |

**Tableau 24. Most frequently reported serious ADR associated with ivermectin for each System Organ Class (SOC) in SSA**

If several sADRs belonging to the same SOC are reported in a single patient (ICSR form), the SOC is counted only once in the total.

| SOC (number of serious ADR reports) | Most frequently reported serious ADR | | | | |
|---|---|---|---|---|---|
| | 1st reported (n) | 2nd reported (n) | 3rd reported (n) | 4th reported (n) | 5th reported (n) |
| **General disorders and administration site conditions (176)** | Drug ineffective (38) | Pyrexia (21) | Asthenia (17) | Drug interaction (12) | Fatigue (12) |
| **Skin and subcutaneous tissue disorders (131)** | Pruritus (29) | Rash (22) | Erythema (15) | Toxic epidermal necrolysis (14) | Rash maculo-papular (13) |
| **Nervous system disorders (97)** | Headache (23) | Dizziness (14) | Convulsion (10) | Loss of consciousness (9) | Sleepiness (8) |
| **Gastrointestinal disorders (78)** | Vomiting (18) | Abdominal pain (15) | Nausea (13) | Diarrhea (9à | Dysphagia (7) |
| **Infections and infestations (77)** | Strongyloidiasis (34) | Pneumonia (11) | Sepsis (6) | Acarodermatitis (5) | Conjunctivitis (4) |
| **Injury, poisoning, procedural complications (67)** | Off label use (15) | Accidental exposure (10) | Incorrect route of administration (6) | Exposure during pregnancy (4) | Product use in unapproved indication (4) |
| **Investigations (56)** | Weight decreased (5) | Hepatic enzyme increased (5) | Platelet count decreased (4) | CRP increased (3) | Heart rate increased (3) |
| **Respiratory, thoracic, mediastinal disorders (52)** | Dyspnea (18) | ARDS (7) | Respiratory failure (5) | Asthma (4) | Cough (3) |
| **Hepatobiliary disorders (47)** | Hepatitis (10) | Hepatocellular injury (9) | Liver disorder (5) | Hepatic failure (4) | Abnormal hepatic function (4) |
| **Psychiatric disorders (42)** | Confusional state (13) | Hallucination (4) | Agitation (3) | Delirium (3) | Depression (3) |
| **Blood and lymphatic system disorders (42)** | Eosinophilia (18) | Lymphadenopathy (5) | Agranulocytosis (4) | Anemia (4) | Thrombocytopenia (4) |
| **Renal and urinal disorders (29)** | Acute kidney injury (7) | Hematuria (3) | Kidney failure (3) | Abnormal kidney function (3) | Anuria (2) |
| **Musculoskeletal, connectives tissues disorders (26)** | Pain in extremity (8) | Arthralgia (7) | Back pain (6) | Myalgia (6) | Musculoskeletal weakness (3) |
| **Cardiac disorders (27)** | Cardiac arrest (5) | Tachycardia (5) | Palpitations (4) | Cardiac failure (4) | Cardio-respiratory arrest (3) |
| **Vascular disorders (24)** | Hypotension (4) | Circulatory collapse (2) | Hematoma (2) | Hypertension (2) | Orthostatic hypotension (2) |
| **Metabolism and nutrition disorders (24)** | Decreased appetite (5) | Hyperkaliemia (4) | Dehydration (3) | Hyponatremia (3) | Hyperglycemia (2) |
| **Eye disorders (21)** | Blindness (3) | Vision blurred (3) | Visual impairment (3) | Conjunctival haemorrhage (2) | Ocular irritation (2) |
| **Immune system disorders (13)** | Hypersensitivity (6) | Anaphylactic shock (3) | Anaphylactic reaction (1) | Drug hypersensitivity (1) | Immune system disorder (1) |
| **Pregnancy, puerperium, perinatal disorders (9)** | Abortion spontaneous (4) | Foetal death (2) | Oligohydramnios (1) | Premature delivery (1) | Stillbirth (1) |
| **Neoplasm benign, malignant, unspecified (8)** | Hodgkin's disease (3) | Hydatidiform mole (1) | Large B-cell lymphoma (1) | Glioblastoma (1) | T-cell lymphoma (1) |
| **Ear and labyrinth disorders (8)** | Vertigo (5) | Tinnitus (2) | Deafness (1) | Ototoxicity (1) | |
| **Endocrine disorders (7)** | Hypothyroidism (2) | Adrenal insufficiency (1) | Diabetes insipidus (1) | Adrenocortical insufficiency (1) | Autoimmune thyroiditis (1) |
| **Social circumstances (4)** | Loss of independence (2) | Immobile (1) | Impaired driving ability (1) | | |
| **Surgical and medical procedures (4)** | Central catheterization (1) | Limb operation (1) | Spinal operation (1) | Mechanical ventilation (1) | Endotracheal intubation (1) |
| **Reproductive system and breast disorders (3)** | Menstruation irregular (1) | Scrotal oedema (1) | Penile swelling (1) | Scrotum swelling (1) | |
| **Product issues (3)** | Product taste abnormal (1) | Wrong label (1) | Product substitution issue (1) | Product availability issue (1) | |
| **Congenital, familial and genetic disorders (1)** | Congenital anomaly (1) | | | | |

**Tableau 25. Most frequently reported serious ADR associated with ivermectin for each System Organ Class (SOC) in RoW**

If several sADRs belonging to the same SOC are reported in a single patient (ICSR form), the SOC is counted only once in the total.

| **Suspected sADRs**, n (%) | **Sub-Saharan sADRs** | **RoW sADRs** | **Total sADRs** |
|---|---|---|---|
| Headaches | 60 (73.2%) | 22 (26.8%) | 82 |
| Asthenia | 61 (78.2%) | 17 (21.8%) | 78 |
| Pruritus | 47 (61.8%) | 29 (38.2%) | 76 |
| Pyrexia | 42 (66.7%) | 21 (33.3%) | 63 |
| Coma | 31 (86.1%) | 5 (13.9%) | 36 |
| Dizziness | 29 (67.4%) | 14 (32.6%) | 43 |
| Vomiting | 16 (50.0%) | 16 (50.0%) | 34 |
| Rash | 9 (29.0%) | 22 (71.0%) | 31 |
| Diarrhea | 20 (66.7%) | 10 (33.3%) | 30 |

**Tableau 26. Frequency of ten most reported suspected sADRs by regions and in total**

The syndromes of interest which occurred after ivermectin intake and described in Table 20 are reported in Table 27.

| **Suspected syndromes**, n (%) | **Sub-Saharan cases** | **RoW cases** | **Total cases** |
|---|---|---|---|
| Encephalopathy | 32 (58.2%) | 23 (41.8%) | 55 |
| Confusional disorders | 6 (27.3%) | 16 (72.7%) | 22 |
| Toxidermia | 8 (24.2%) | 25 (75.8%) | 33 |
| Psychotic disorders | 1 (9.1%) | 10 (90.9%) | 11 |
| Renal disorders | 1 (5.3%) | 18 (94.7%) | 19 |
| Hepatic disorders | 2 (3.8%) | 51 (96.2%) | 53 |
| Mazzotti reaction | 34 (81.0%) | 8 (19.0%) | 42 |

**Tableau 27. Frequency of reported suspected syndromes by regions and in total**

Ivermectin indications for the 23 serious encephalopathies which occurred outside SSA were scabies (8), acarodermatitis (4), strongyloidiasis (4), rosacea (1), onchocerciasis (1) and unknown indications (5). Ivermectin indications for the 32 serious encephalopathies which occurred in SSA were onchocerciasis (30), unspecified filariasis (1) and unknown (1). Indications for ivermectin treatment in cases of serious toxidermia were scabies (9), unknown (12), strongyloidiasis (3), lice (3), acarodermatitis (2), cysticercosis (1), onchocerciasis (1), unspecified filariasis (1) and in one case ivermectin had been administered erroneously. Ivermectin

indications for cases of serious Mazzotti reactions were onchocerciasis (28), lice (3), parasitosis (1), strongyloidiasis (1), worms (1), filariasis (1) and not reported (7).

#### *4.4.4.2 Disproportionality analysis*

The results of the disproportionality analyses of sADRs of interest as well as non-cases after administration of ivermectin compared to benzimidazole drugs are presented in Table 28. After adjustment, the relative frequency of serious headaches reported after treatment with ivermectin and with benzimidazole drugs was similar (adjusted ROR [aROR]: 1.22, 95% CI: 0.83-1.78). This was also the case in origin-stratified analysis (aROR: 1.16, 95% CI: 0.68-1.98 and aROR: 1.39, 95% CI: 0.76-2.53 in SSA and RoW, respectively). In contrast, serious encephalopathies were much more frequently reported after ivermectin than benzimidazole treatment, globally (aROR: 9.23, 95% CI: 4.56-18.61), in SSA countries (aROR: 27.1, 95% CI: 6.34-116.1) and in the RoW (aROR: 6.30, 95% CI: 2.68-14.8). Reports of confusional disorders were strongly associated with ivermectin use globally (aROR: 4.05, 95% CI: 1.81-9.09), in the RoW (aROR: 3.66, 95% CI: 1.49-8.87) but not in SSA (aROR: 3.87, 95% CI: 0.76-19.6). Serious seizures were not more frequently reported after ivermectin than after benzimidazole drugs (aROR: 0.49, 95% CI: 0.49-0.97).

Drug Reaction with Eosinophilia and Systemic Symptoms (DRESS) was more frequently reported with ivermectin than with benzimidazole drugs (aROR: 8.59, 95% CI: 1.85-39.9). No adjustments were possible for the analysis of DRESS because of the low number of cases. Serious toxidermias (DRESS, Stevens-Johnson syndrome, toxic epidermal necrolysis and acute generalized exanthematous pustulosis) were more frequently reported with ivermectin than with benzimidazole drugs globally (aROR: 4.43, 95% CI: 2.07-9.47) and in the RoW (aROR: 6.05, 95% CI: 2.76-13.3), but not in SSA countries (aROR: 0.52, 95% CI: 0.05-5.01). It is noticeable that eight cases of toxidermia were excluded from the analyses because ivermectin was co-administered with albendazole.

Serious psychotic disorders and suicidal disorders were not more frequently reported with ivermectin than with benzimidazole drugs (aROR: 1.78, 95% CI: 0.70-4.53 and aROR: 7.67, 95% CI: 0.85-69.0, respectively).

Only 5 cases of Severe Acute Respiratory Syndrome (SARS) were reported, and no significant associations were found. aROR values did not indicate any associations for serious

hepatic disorders or serious renal disorders either (aROR: 0.51, 95% CI: 0.36-0.74 and aROR: 1.36, 95% CI: 0.66-2.85, respectively).

Serious cardiac failures were significantly associated with ivermectin compared to benzimidazole drug intake (ROR: 11.4, 95% CI: 1.37-94.9, no adjustment possible). Serious rhythm disorders were not found to be associated with ivermectin compared to benzimidazole drugs.

Finally, serious Mazzotti reactions were strongly associated with ivermectin compared to benzimidazole drugs both in SSA (aROR: 1.95, 95% CI: 1.09-3.52) and in the RoW (aROR: 19.7, 95% CI: 2.20-175.5).

#### *4.4.4.3 Sensitivity analysis*

Disproportionality analyses were repeated with all antinematodal drugs rather than only benzimidazole drugs as control group (Table 29). Associations for reports of serious headache in RoW (aROR: 1.82, 95% CI: 1.01–3.28) and serious rhythm disorders in RoW (aROR: 3.45, 95% CI: 1.02–11.7) were strengthened in these sensitivity analyses. No changes were found for encephalopathies, confusional disorders, DRESS, toxidermias, seizures, renal disorders, suicidal disorders, psychiatric disorders, SARS, hepatic disorders and Mazzotti reactions. In contrast to the primary analysis, the sensitivity analysis identified no association for cardiac failures.

| Serious adverse drug reaction | Drugs | Cases | Non-cases | Crude ROR (95% CI) | Adjusted ROR [a] (95% CI) | Adjusted ROR [b] in Sub-Saharan Africa (95% CI) | Adjusted ROR [b] in RoW (95% CI) |
|---|---|---|---|---|---|---|---|
| Headache | **Ivermectin** | 71 | 502 | 1.40 ** (1.01–1.93) | 1.22 (0.83–1.78) | 1.16 (0.68–1.98) | 1.39 (0.76–2.53) |
| | Benzimidazoles | 99 | 980 | Ref. | Ref. | Ref. | Ref. |
| Encephalopathy | **Ivermectin** | 55 | 518 | 10.3 *** (5.35–19.9) | 9.23 *** (4.56–18.6) | 27.1 *** (6.34–116.1) | 6.30 *** (2.68–14.8) |
| | Benzimidazoles | 11 | 1068 | Ref. | Ref. | Ref. | Ref. |
| Confusional disorders | **Ivermectin** | 22 | 551 | 3.88 *** (1.87–8.05) | 4.05 *** (1.81–9.09) | 3.87 (0.76–19.6) | 3.66 *** (1.49–8.97) |
| | Benzimidazoles | 11 | 1068 | Ref. | Ref. | Ref. | Ref. |
| Seizure | **Ivermectin** | 11 | 562 | 0.49 (0.25–0.97) | 0.83 (0.40–1.72) | N/A | N/A |
| | Benzimidazoles | 41 | 1038 | Ref. | Ref. | | |
| DRESS * | **Ivermectin** | 9 | 564 | 8.59 *** (1.85–39.9) | N/A | N/A | N/A |
| | Benzimidazoles | 2 | 1072 | Ref. | | | |
| Toxidermia | **Ivermectin** | 25 | 548 | 3.47 *** (1.79–6.73) | 4.43 *** (2.07–9.47) | 0.52 (0.05–5.01) | 6.05*** (2.76–13.3) |
| | Benzimidazoles | 15 | 1065 | Ref. | Ref. | Ref. | Ref. |
| Psychotic disorders | **Ivermectin** | 10 | 563 | 1.72 (0.73–4.08) | 1.78 (0.70–4.53) | N/A | 1.62 (0.62–4.23) |
| | Benzimidazoles | 11 | 1068 | Ref. | Ref. | | Ref. |
| Suicidal behavior | **Ivermectin** | 4 | 569 | 7.58 * (0.84–68.0) | 7.67 * (0.85–69.0) | N/A | N/A |
| | Benzimidazoles | 1 | 1078 | Ref. | Ref. | | |
| SARS ** | **Ivermectin** | 4 | 569 | 7.58 * (0.84–68.0) | N/A | N/A | N/A |
| | Benzimidazoles | 1 | 1078 | Ref. | | | |
| Renal disorders | **Ivermectin** | 17 | 556 | 2.03 ** (1.02–4.05) | 1.36 (0.66–2.85) | N/A | N/A |
| | Benzimidazoles | 16 | 1063 | Ref. | Ref. | | |
| Hepatic disorders | **Ivermectin** | 50 | 523 | 0.61 (0.44–0.86) | 0.51 (0.36–0.74) | 1.36 (0.08–21.9) | 0.50 (0.35–0.73) |
| | Benzimidazoles | 145 | 934 | Ref. | Ref. | Ref. | Ref. |
| Cardiac failure | **Ivermectin** | 6 | 567 | 11.4 ** (1.37–94.9) | N/A | N/A | N/A |
| | Benzimidazoles | 1 | 1078 | Ref. | | | |
| Rhythm disorders | **Ivermectin** | 7 | 566 | 3.32 * (0.97–11.40) | 3.18 * (0.86–11.7) | N/A | 3.18 * (0.86–11.7) |
| | Benzimidazoles | 4 | 1075 | Ref. | Ref. | | Ref. |
| Mazzotti's reaction | **Ivermectin** | 36 | 537 | 2.94 *** (1.74–4.99) | 2.16 ** (1.16–4.03) | 1.95 ** (1.09–3.52) | 19.7 *** (2.20–175.5) |
| | Benzimidazoles | 24 | 1055 | Ref. | Ref. | Ref. | Ref. |

**Tableau 28. Disproportionality analysis of serious adverse reactions associated with ivermectin compared to benzimidazole drugs**

[a] Adjusted for origin (Sub-Saharan Africa or RoW), gender, age and period of notification; [b] Adjusted for gender, age and period of notification

* Drug reaction with eosinophilia and systemic symptoms; ** Severe Acute Respiratory Syndrome.

| Severe adverse drug reaction | Drugs | Cases | Non-cases | Crude ROR (95% CI) | Adjusted ROR [a] (95% CI) | Adjusted ROR [b] in Sub-Saharan Africa (95% CI) | Adjusted ROR [b] in RoW (95% CI) |
|---|---|---|---|---|---|---|---|
| Headache | **Ivermectin** | 71 | 502 | 1.64 *** (1.20–2.25) | 1.45 ** (1.00–2.11) | 1.31 (0.78–2.20) | 1.82 ** (1.01–3.28) |
| | Benzimidazoles | 109 | 1265 | 1 [Reference] | 1 [Reference] | 1 [Reference] | 1 [Reference] |
| Encephalopathy | **Ivermectin** | 55 | 518 | 9.01 *** (5.12–15.9) | 7.60 *** (4.13–14.0) | 31.6 *** (7.37–135.3) | 4.70 *** (2.25–9.81) |
| | Benzimidazoles | 16 | 1358 | 1 [Reference] | 1 [Reference] | 1 [Reference] | 1 [Reference] |
| Confusional disorders | **Ivermectin** | 22 | 551 | 3.39 *** (1.77–6.50) | 3.27 *** (1.60–6.69) | 4.82 * (0.96–24.2) | 2.81 *** (1.28–6.18) |
| | Benzimidazoles | 16 | 1358 | 1 [Reference] | 1 [Reference] | 1 [Reference] | 1 [Reference] |
| Seizure | **Ivermectin** | 11 | 562 | 0.59 (0.30–1.15) | 0.88 (0.43–1.82) | 0.13 (0.02–1.12) | 1.13 (0.52–2.42) |
| | Benzimidazoles | 44 | 1330 | 1 [Reference] | 1 [Reference] | 1 [Reference] | 1 [Reference] |
| DRESS * | **Ivermectin** | 9 | 564 | 10.9 *** (2.36–50.8) | N/A | N/A | N/A |
| | Benzimidazoles | 2 | 1372 | 1 [Reference] | | | |
| Toxidermia | **Ivermectin** | 25 | 548 | 4.13 *** (2.16–7.90) | 5.41 *** (2.58–11.3) | 0.41 (0.04–4.09) | 4.87*** (2.17–10.9) |
| | Benzimidazoles | 15 | 1359 | 1 [Reference] | 1 [Reference] | 1 [Reference] | 1 [Reference] |
| Psychotic disorders | **Ivermectin** | 10 | 563 | 1.51 (0.68–3.34) | 1.48 (0.63–3.48) | N/A | 1.51 (0.62–3.71) |
| | Benzimidazoles | 16 | 1358 | 1 [Reference] | 1 [Reference] | | 1 [Reference] |
| Suicidal behavior | **Ivermectin** | 4 | 569 | 0.64 (0.21–1.93) | 0.61 * (0.20–1.85) | N/A | N/A |
| | Benzimidazoles | 15 | 1359 | 1 [Reference] | 1 [Reference] | | |
| SARS ** | **Ivermectin** | 4 | 569 | 10.2 ** (1.13–91.8) | N/A | N/A | N/A |
| | Benzimidazoles | 1 | 1373 | 1 [Reference] | | | |
| Renal disorders | **Ivermectin** | 17 | 556 | 1.88 * (0.99–3.56) | 1.37 (0.69–2.69) | N/A | N/A |
| | Benzimidazoles | 22 | 1352 | 1 [Reference] | 1 [Reference] | | |
| Hepatic disorders | **Ivermectin** | 50 | 523 | 0.78 (0.56–1.10) | 0.71 (0.50–1.02) | 1.35 (0.10–25.1) | 0.70 (0.49–1.02) |
| | Benzimidazoles | 149 | 1225 | 1 [Reference] | 1 [Reference] | 1 [Reference] | 1 [Reference] |
| Cardiac failure | **Ivermectin** | 6 | 567 | 2.79 * (0.83–9.38) | N/A | N/A | N/A |
| | Benzimidazoles | 1 | 1373 | 1 [Reference] | | | |
| Rhythm disorders | **Ivermectin** | 7 | 566 | 3.39 * (1.07–10.7) | 2.79 * (0.83–9.38) | N/A | 3.45 ** (1.02–11.7) |
| | Benzimidazoles | 5 | 1369 | 1 [Reference] | 1 [Reference] | | 1 [Reference] |
| Mazzotti's reaction | **Ivermectin** | 36 | 537 | 3.62 *** (2.15–6.08) | 2.61 *** (1.41–4.85) | 2.17 *** (1.22–3.88) | 16.5 *** (1.98–137.4) |
| | Benzimidazoles | 25 | 1349 | 1 [Reference] | 1 [Reference] | 1 [Reference] | 1 [Reference] |

**Tableau 29. Disproportionality analysis of serious adverse reactions associated with ivermectin compared to other antinematodal drugs**

[a] Adjusted for origin (Sub-Saharan Africa or RoW), gender, age and period of notification; [b] Adjusted for gender, age and period of notification;

* Drug reaction with eosinophilia and systemic symptoms; ** Severe Acute Respiratory Syndrome.

### 4.4.5 Discussion

Our study used a case-non-case approach to assess the association between the use of ivermectin and the reporting of neurological as well as non-neurological suspected sADRs, recorded in the WHO drug adverse events database from 2003 to 2020. To our knowledge, it is the first to globally review the main serious ADRs reported with ivermectin. Some strong significant disproportionality signals were found, showing more frequent reporting of encephalopathies after ivermectin than after benzimidazoles, both in SSA countries and in the RoW. Disproportionality signals were also identified for serious toxidermias, serious confusional disorders and serious Mazzotti reactions with ivermectin when compared with benzimidazole drugs or all non-ivermectin antinematodal drugs. A less consistent signal was found for cardiac failures and further studies are needed to confirm this result.

Some of these results were expected, given the different mechanisms of action of ivermectin and benzimidazoles on the various targeted parasites. Ivermectin exerts a strong microfilaricidal effect on filariae, leading to a destruction of mf within one week after treatment. In subjects infected with *Onchocerca volvulus*, the destruction of mf in the skin is associated with inflammatory processes leading to the so-called Mazzotti reaction. In those infected with *L. loa*, the drug probably induces a paralysis of the *L. loa* mf, which are then drained passively in the blood circulation. If the microfilarial density is high, the process can lead to an embolization of mf in the brain capillaries, to inflammatory reactions at the cerebral level, and to an encephalopathy. In contrast, benzimidazoles have little short-term effect on the mf of any filarial species, and thus do not induce Mazzotti reactions, but impair the production of new mf by the adult female worms.

The US Food and Drug Administration (FDA) approved product information for ivermectin mentions that "Rarely, patients with onchocerciasis who are also heavily infected with *Loa loa* may develop a serious or even fatal encephalopathy either spontaneously or following treatment with an effective microfilaricide." (FDA n.d.) In our study, we confirmed the findings of a previous analysis of the data in VigiBase (Chandler 2018) which identified encephalopathies reported with ivermectin also outside of SSA where *L. loa* is not endemic. In addition, we quantified this association by estimating aRORs for ivermectin-induced encephalopathy. aROR was higher in the

SSA countries than in the RoW but both were significant, demonstrating a strong global safety signal. Another recent publication described the case of a 13 years old boy presenting a progressive encephalopathy after a single oral dose of ivermectin given at 230 µg per kg, i.e. only slightly higher than the dose used for ivermectin mass drug administration for onchocerciasis (150 µg/kg) and lymphatic filariasis (200 µg/kg) control or to prevent scabies infection (200 µg/kg). The authors found that the patient was a carrier of non-sense mutations in the gene coding for the ATP-binding cassette subfamily B member 1 (ABCB1) transporter which is known to efflux ivermectin from the brain. These mutations can lead to neurological adverse reactions induced by ivermectin (Baudou et al. 2020). Our results are therefore consistent with the literature and support the evidence of post-ivermectin serious neurological ADRs in some people not infected with *L. loa*. The clinical presentations of *Loa*-related and non-*Loa*-related post-ivermectin neurological ADRs are summarized in Table 30.

The FDA-approved product information for ivermectin also includes the risk of toxic epidermal necrolysis and Stevens-Johnson syndrome as very rare events. By identifying a strong disproportionality signal between ivermectin and benzimidazole drugs or all other antinematodal drugs, our study suggests that ivermectin may be associated with a higher risk of toxidermias than other antinematodal drugs. We also found in VigiBase two types of toxidermia that were never mentioned in the literature: 9 cases of DRESS and 2 cases of acute generalized exanthematous pustulosis after ivermectin intake. These findings could be of great interest for clinicians considering ivermectin treatment in patients at risk for these sADRs or assessing the causality of ivermectin in the development of a toxidermia.

Our study has several strengths. First, we used the global ADRs database VigiBase intended to collect information on suspected ADRs from nearly all national pharmacovigilance centers in the world, allowing us to estimate ROR for rare events with sufficient statistical power and to stratify on geographical origin (SSA vs. RoW). Second, analyses were performed with adjustment for several potential confounders such as origin, gender, age and period of notification. Third, nearly all results of our principal analysis were confirmed in our sensitivity analyses considering all antinematodal agents. Fourth, our results are consistent with already known risk associated with ivermectin (encephalopathy in SSA and Mazzotti reactions).

Limitations of this study include the concern about under-reporting of suspected ADRs and differences in the under-reporting between different countries as well as the lack of information on the number of drug administrations, which is a major disadvantage inherent in

studies using pharmacovigilance databases (Bégaud et al. 2002; Van Der Heijden et al. 2002). Although under-reporting may be less important since we focused on serious ADRs (which are more likely to be reported) (Martin et al. 1998), our analyses cannot measure the real risk of ADR but only the differences in reported events. Indeed, subjects in the control group (non-cases) are not healthy controls but patients with other various reported ADRs and pharmacovigilance data do not consider the total amount of patients exposed to the drug. Nevertheless, there is no apparent reason that, in a specific region, ADRs would be more or less reported with ivermectin than those occurring after treatment with benzimidazole drugs or other antinematodal drugs. By analyzing real-life surveillance data, disproportionality analyses have demonstrated their usefulness for detecting drug risks (Maciá-Martínez et al. 2016; Montastruc et al. 2011). Anyway, these results should be taken with caution because of potential missing information. Pharmacovigilance systems are not yet well established in SSA countries. In 2017, only 30% of these countries had specific procedures for the monitoring of ADRs and only 28% had a platform for coordinating pharmacovigilance activities at the national level (Kaboré et al. 2017). Cases of serious adverse events occurring during the ivermectin mass drug administration organized by the onchocerciasis and LF control programs have to be reported by the countries to the Mectizan Donation Program, but the extent to which all relevant observations are recorded in the rural areas where onchocerciasis and LF are endemic and then passed on to the central level is unknown as is the extent to which they are reported into the WHO VigiBase. For example, we found no cases from Cameroon even though ivermectin mass drug administration programs have been ongoing there for 30 years, and many cases are known to have occurred since the early 1990s (Twum-Danso 2003). In addition, the first case of a post-ivermectin ADR was reported in VigiBase in December 2003 while the first reported death after ivermectin was reported by the WHO Drug Information in 1991 (Anonymous 1991). We consider it likely that availability of complete data from SSA would show more cases associated with ivermectin use and would increase the strength of the safety signals we identified.

In addition, a notoriety bias (selection bias in which a case has a greater chance of being reported if the drug is known to cause, thought to cause, or likely to cause the event of interest (Pariente et al. 2007)) could be considered for reports of encephalopathy in SSA given that the first cases of encephalopathies involving ivermectin led to complications in the early mass drug administration campaigns for elimination of onchocerciasis. However, it is unlikely that such bias exist for two reasons (i) in SSA countries, ivermectin is distributed as part of mass treatment

organized by the Ministries of Health, and those of *L. loa*-endemic countries might be less inclined to report post-ivermectin sADRs because the cases are not regarded as exceptional and (ii) we also found a strong disproportionality signal in the RoW which is not being affected by this bias.

Our analyses identified serious ADRs that can be associated with ivermectin use that to date have received little, if any attention. As ivermectin is currently widely used off label, especially in Latin America, to control COVID-19 without strong evidence for beneficial effect (Mega 2020), this study is timely to describe the various suspected sADRs to which this population is potentially exposed even in the absence of onchocerciasis and loiasis endemicity. While ivermectin's excellent safety profile is the basis for mass drug administration campaigns and progress towards elimination in particular of onchocerciasis, one must remain aware and vigilant about the sADRs it may possibly induce.

| | Main risk factors | Main symptoms | Main mechanisms involved |
|---|---|---|---|
| **Possible/Probable *Loa loa* encephalopathy temporally related to Mectizan (PLERM)** | - Intensity of the initial *Loa* microfilaremia | - *12-24h following treatment*: fever, fatigue, arthralgia, agitation, mutism, incontinence<br>- *24-72h following treatment*: consciousness disorders including coma and extrapyramidal signs, typical hemorrhages in the palpebral conjunctiva, retinal lesions<br>- Existence of diffuse pathological process at electroencephalogram level | - Paralysis of the microfilariae due to the action of ivermectin resulting in embolisms in the brain capillaries<br>- Inflammatory processes at the cerebral level |
| **Other encephalopathies related to ivermectin:**<br>- **Toxicosis due to an overdose**<br>- **Toxicosis due to a mutation** | - Polymorphism of MDR1 gene<br>- Deficiency in P-glycoproteins<br>- Intentional or unintentional overdosing | - *Few hours after administration*: nausea, vomiting, abdominal pain, salivation, tachycardia, hypotension, ataxia, pyramidal signs, binocular diplopia<br>- Normal paraclinical tests results | - Passage of ivermectin through the blood-brain barrier (due to overdose or mutation of transporters/metabolism actors) |

**Tableau 30. Possible/Probable *Loa loa* encephalopathy temporally related to Mectizan and other encephalopathies related to ivermectin: mains risk factors, symptoms and mechanisms involved**

### 4.4.6 Conclusion

**Cette étude montre que les effets indésirables graves rapportés dans la base mondiale de pharmacovigilance de l'OMS associés à l'ivermectine sont cohérents avec ceux mentionnés dans le résumé des caractéristiques du produit (RCP), mais fournit également de nouveaux signaux. Des encéphalopathies graves post-ivermectine peuvent également survenir en dehors des régions endémiques de *L. loa* mais la compréhension du mécanisme par lequel elles surviennent nécessite des études complémentaires. Un nouveau signal concernant deux toxidermies graves, le syndrome DRESS et la pustulose exanthématique aiguë généralisée, est également décrit. Enfin, un manque de notification des effets indésirables des médicaments est décrit dans certains pays d'Afrique Sub-Saharienne nécessitant des actions pour augmenter les taux de notification dans ces pays.**

# 5. Évaluation du lévamisole dans la prise en charge de la microfilarémie à *Loa loa* et étude systématique de ses effets indésirables

## 5.1 Généralités

Le lévamisole est une molécule synthétique, isomère du tétramisole (Figure 21), qui a été décrite pour la première fois en 1966 (Thienpont et al. 1966). Prescrit initialement comme antihelminthique vétérinaire, puis humain (Miller 1980), elle fait partie de la liste des médicaments essentiels de l'OMS depuis 1988 (World Health Organization 2019).


**Figure 21. Structure chimique du lévamisole**

Par la suite, le lévamisole a démontré des propriétés immunomodulatrices qui ont permis son utilisation en cancérologie, en tant qu'adjuvant potentialisateur de chimiothérapies, ainsi que dans certaines maladies auto-immunes. Finalement, depuis 2009, le lévamisole a été détourné et est maintenant largement mésusé en tant que produit de coupe de la cocaïne à cause, notamment, de son prix peu élevé et de ses propriétés chimiques empêchant sa détection dans les tests de pureté de rue (Karch et al. 2012).

Depuis 1999, des rapports de pharmacovigilance ont mis en cause le lévamisole dans l'apparition d'effets secondaires graves indésirables et, par conséquent, son AMM a été supprimée en Europe et en Amérique du Nord. En revanche, dans toutes les autres régions du monde, on continue à utiliser des doses uniques de lévamisole comme traitement antihelminthique.

Afin de mettre en évidence des signaux associant un des modes d'utilisation à la survenue d'effets indésirables spécifiques, nous avons réalisé une étude de pharmacovigilance à partir de la base de pharmacovigilance mondiale de l'OMS.

## 5.2 Effets indésirables

### Adverse reactions with levamisole vary according to its indications and misuse: a systematic pharmacovigilance study

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**Running head**: Levamisole adverse drug reactions

**Keywords:** Levamisole, Adverse drug reactions, Disproportionality, Pharmacovigilance

**What is already known about this subject?**

- Levamisole has had many different indications, has been misused and has been associated in the occurrence of serious adverse drug reactions (ADRs).
- This association has led several countries to suspend its use. Nevertheless, other countries still use it daily for its antiparasitic indication and do not report serious ADRs.

**What this study adds?**

- The majority of levamisole-related ADRs concerns its immunomodulatory proprieties.
- Single dose treatments of levamisole for an antiparasitic indication appear to have a good safety profile.
- The use of levamisole in specific areas where benzimidazole resistance is feared could be an important resource to overcome the possible occurrence of resistance.

### 5.2.1 Abstract

**Aim.** Levamisole was initially prescribed for the treatment of intestinal worms. Because of immunomodulatory properties, levamisole has been used in inflammatory pathologies and in cancers in association with 5-fluorouracil. Levamisole is misused as a cocaine adulterant. Post-marketing reports have implicated levamisole in the occurrence of adverse drug reactions (ADRs) and its use is now limited in Europe and North America. In contrast, all other parts of the World continue to use single-dose as an anthelmintic. The aim of this study was to identify ADRs reported after levamisole exposure in VigiBase, the WHO's pharmacovigilance database, and analyze their frequency compared to other drugs and according to levamisole type of use.

**Methods**. All levamisole-related ADRs were extracted from VigiBase®. Disproportionality analyses were conducted to investigate psychiatric, hepatobiliary, renal, vascular, nervous, blood, skin, cardiac, musculoskeletal and general ADRs associated with levamisole and other

drugs exposure. In secondary analyses, we compared the frequency of ADRs between levamisole and mebendazole and between levamisole type of use.

**Results**. Among the 1763 levamisole-related ADRs identified, psychiatric disorders (Reporting Odds-Ratio with 95% confidence intervals: 1.4 [1.2-2.6]), hepatobiliary disorders (2.4 [1.9-4.3]), vasculitis (6.5 [4.1-10.6]), encephalopathy (22.5 [17.4-39.9]), neuropathy (4.3 [2.9-7.1]), hematological disorders, mild rashes and musculoskeletal disorders were more frequently reported with levamisole than with other drug. The majority of levamisole-related ADRs occurred when the drug was administrated for a non-anti-infectious indication.

**Conclusion**. The great majority of the levamisole-related ADRs concerned its immunomodulatory indication and multiple doses regimen. Our results suggest that single-dose treatments for anthelmintic action have a good safety profile.

### 5.2.2 Introduction

Levamisole is an old drug derived from imidazothiazole, discovered in 1966 and originally used in veterinary medicine as an anthelmintic and then marketed for the same indication in humans. The anthelminthic action of levamisole is mainly used against *Ascaris lumbricoides*, *Trichuris trichiura*, and hookworms (*Necator americanus* and *Ancylostoma duodenale*), the three main soil-transmitted helminths (STH) belonging to the WHO's list of Neglected Tropical Diseases (NTD). Because of levamisole's broad spectrum activity and safety, as well as the fact that it is relatively inexpensive and requires only a single oral dose to treat STH, it has been included in the WHO list of essential medicines in 1988 (World Health Organization 2019). From the early 1980s, national programs to control STH have typically implemented annual mass drug administration with any of the following anthelmintic drugs: albendazole, mebendazole, levamisole or pyrantel (the exact drug is at the discretion of each country) (World Health Organization 2006). However, since 2003, the three drugs used by national programs are albendazole, mebendazole and praziquantel (only in combination with one of the other two treatments) while levamisole is no longer used in mass drug administration according to the WHO data (World Health Organization n.d., 2020). This is due to three main reasons: (i) contrarily to levamisole, benzimidazoles (especially mebendazole) are very little absorbed by the organism and remain in the intestine where they kill the intestinal parasites, which is a guarantee of safety;

(ii) since 2010, two pharmaceutical companies have been donating large quantities of mebendazole (Johnson & Johnson) and albendazole (GlaxoSmithKline) to countries where STH are endemic, thus promoting the use of these molecules from an economic perspective; (iii) albendazole and mebendazole do not require weight adjustment, unlike levamisole which is used at 2.5 mg/kg. In 2008, Albonico *et al.* reports that "no literature was found specifically on the use of levamisole in pre-school age children" (i.e. as part of mass drug administration for STH infections) (Albonico et al. 2008). However, it appears that the last documented uses of levamisole in national control programs were in China, Iran, Vietnam, Brazil, Kenya and Nigeria in the 1990s (Albonico et al. 2008; Urbani and Albonico 2003).

Besides these national control programs, levamisole can be purchased with or without a medical prescription for personal use in many countries around the world, particularly in areas where STH are highly endemic (South America, Asia and Africa). For both individual treatment and mass drug administration, levamisole is usually administered as a single oral dose of 2.5 mg/kg or 80 mg for all school-age children to treat STH (World Health Organization 2011).

The mechanisms of action of levamisole are multiple and not yet fully elucidated. Levamisole is able to paralyze nematode muscles, leaving the worms unable to attach themselves to the mucous membranes, and causing them to be expelled through the intestine (Martin 1993). Levamisole has several other effects on human organisms: it exerts immunomodulatory properties and acts on the dopaminergic, cholinergic and noradrenergic systems (Renoux 1980). Levamisole was subsequently used for its immunomodulatory action in certain forms of rheumatoid arthritis and in association with 5-fluorouracil in patients with colon cancer or melanoma (Moertel et al. 1990). In some countries, levamisole is also used in the treatment of pediatric nephrotic syndrome (Mühlig et al. 2019).

The majority of levamisole-related adverse drug reactions (ADRs) reported when used as an anthelmintic treatment were mild and transient (Albonico et al. 2003; Gatti et al. 1972; Lionel et al. 1969; Nagaty et al. 1978). During the period 1994-2000, some cases of nervous system disorders have been reported in North Vietnam but levamisole was produced locally, which, according to the Centre for Adverse Drug Reaction of the Vietnam ministry of health, "raises the issue of quality assurance" (Urbani and Albonico 2003). In 2009, for the first time, 16 cases of multifocal inflammatory leukoencephalopathy were reported from China after a single dose of levamisole (Xu et al. 2009).

The scientific literature also reports various ADRs after levamisole treatment for other purposes than anthelmintic indication. In cancer treatments, levamisole is generally used at high doses (50 mg every 8 hours for 3 days) every 2 weeks during at least 1 year (Mutch and Hutson 1991) and in combination with 5-fluorouracil. Several authors reported cases of multifocal inflammatory leukoencephalopathy (Fassas et al. 1994; Chen et al. 1994; Kimmel and Schutt 1993; Hook et al. 1992; Gordon et al. 1996; Ferroir et al. 1994; Hwang et al. 2003; Luppi et al. 1996; Murray et al. 1997; Figueredo et al. 1995; Piernatale et al. 1996; Mak et al. 2001; Zvi et al. 1998; Kimmel et al. 1995; Critchley et al. 1994; El Kallab et al. 2003; Galassi et al. 1996), vasculitis (Scheinberg et al. 1978), agranulocytosis (Parkinson et al. 1977) and thrombocytopenia (Winquist and Lassam 1995) associated with this regimen.

As part of pediatric nephrotic syndrome or rheumatoid polyarthritis, the recommended dose of levamisole is 2 or 2.5 mg/kg on alternate days for 12–24 months (Mühlig et al. 2019). In studies concerning immunomodulatory properties of levamisole, some serious ADRs have been reported: nervous system disorders (Palcoux et al. 1994), vasculitis (Menni et al. 1997; Macfarlane and Bacon 1978; Rongioletti et al. 1999; Powell et al. 2002; Kirby et al. 1980), and agranulocytosis (Rosenthal 1982; Heyns et al. 1979).

Since 2009, levamisole has been involved in case reports as a cocaine adulterant; the amphetamine-like substance aminorex (an anorectic stimulant) being its metabolite (Karch et al. 2012). Several hypotheses have been made to explain cocaine adulteration with levamisole: its cheapness, the large quantity available, its chemical properties which enable it to go undetected in typically used street purity tests, and/or potentiation of cocaine effects (Raymon and Isenschmid 2009). Severe somatic complications widely reported in users of levamisole-adulterated cocaine include leucopenia, agranulocytosis, leukoencephalopathy, arthritis, thrombotic vasculopathy and vasculitis (Larocque and Hoffman 2012). Cardiac complications, cognitive impairments and cerebral toxicities were also recently described (Gartz et al. 2020; Vonmoos et al. 2018; Allard et al. 2021). As the percentage of levamisole in cocaine powder and the amount of cocaine consumed is never known at the time of consumption, it is very difficult to estimate the level of levamisole exposure in the cases.

Although levamisole is considered as an essential medicine by the WHO, the United States of America (USA) and Europe decided to withdraw its marketing authorization in 2004 and 1998 respectively, and to regulate its use (temporary authorization) for specific indications such as nephrotic syndrome or (as an adjuvant) cancer therapy.

Encephalopathies, vasculitis and agranulocytosis are post-levamisole ADRs which seem related to the type of use of the drug, and thus to the dosage regimen. Besides the adulterated cocaine, information regarding the extent of levamisole use, both in general (including in auto-medication) and specifically for treatment of STH (i.e. at single dose of 2.5 mg/kg) is scarce. As levamisole has been used in many indications, with very different administration schemes and various co-administered drugs, it is likely that the ADRs occurring varies according to each use. The prescription drug information mentions the following ADRs: neutropenia, thrombocytopenia, leukoencephalopathy, hypersensitive reactions, nervousness, sleepiness, depression, nausea, vomiting, reduced appetite, diarrhea, constipation, pancreatitis, skin rash and inflammation, muscle and joint pain, inflammation of the mouth, change of odor (ACE Phamaceuticals 2018). Finally, with the emergence of the COVID-19 pandemic, levamisole has been proposed as a therapeutic strategy option on the basis of its immunomodulatory properties which was thought to improve clinical status of patients with COVID-19 (Roostaei Firozabad et al. 2021). In this context, we searched the WHO global pharmacovigilance database, VigiBase®, for all the suspected ADRs reported after levamisole treatment. We then conducted disproportionality analyses considering the types of use. More specifically, the aims of this study were (i) to identify new pharmacovigilance signals (increased reporting of suspected ADRs after treatment with levamisole compared to other treatments), (ii) to compare ADRs after levamisole according to its type of use (and therefore its regimen), and (iii) to assess, using all available information, the safety of a single dose. Finally, an overview of the known mechanisms of action of levamisole is provided.

## 5.2.3 Methods

### *5.2.3.1 Data source*

Data were extracted from the WHO Global Individual Case Safety Report (ICSR) database VigiBase® (VigiBase n.d.) which includes more than 24 million cases of suspected ADRs reported by national pharmacovigilance centers in more than 130 countries participating in the WHO Program for International Drug Monitoring (Lindquist 2008). An ICSR is an anonymized report for a single individual who experienced adverse event(s) that may be linked to the use of one or

more drugs. ICSR contains sociodemographic information (age, sex, reporter qualification, country of origin, year of report), information about the drug administration (frequency, dosage, co-medications) and information about the reported adverse event(s). The latter includes the seriousness according to the criteria of the International Council for Harmonization of Technical Requirements for Pharmaceuticals for Human Use (ICH) (European Medicines Agency 1998), adverse event verbatim description and associated terms from the Medical Dictionary for Regulatory Activities (MedDRA) developed by the ICH. From Vigibase®, all reports of suspected ADRs associated with levamisole from February 27, 1977 (first ever report of levamisole-related suspected ADR recorded) up to February 7, 2021 were extracted. Primary analysis used all reports from Vigibase®, comparing levamisole-related ADRs to all ADRs reported in the database (any drugs). Mebendazole-related cases were also extracted and used as control cases because of this drug has similar anthelmintic indications. Prior to analysis, suspected duplicate reports identified by an automated screening were excluded (Norén et al. 2007). Suspected ADRs were classified following the MedDRA classification (Brown et al. 1999), grouped at the System Organ Class (SOC) level and at the individual preferred term (PT) level.

#### *5.2.3.2 Study design*

We performed disproportionality analyses using the case/non-case method which allows to identify disproportionate reporting, *i.e.* a higher-than-expected number of adverse reaction reports compared to other reactions recorded in the database by calculating Reporting Odds Ratios (ROR). ROR compares the odds of exposure to levamisole between cases and non-cases (Moore et al. 2005; Puijenbroek et al. 2002).

Cases were defined as reports of each suspected ADR of interest identified by a MedDRA PT. ADRs of interest were identified from the scientific literature or from the drug official information (Summary of Product Characteristics) and include vasculitis, encephalopathies, peripheral neuropathy, convulsions, agranulocytosis, leucopenia, neutropenia, thrombocytopenia, vertigo, fever, tachycardia, failures (cardiac arrest, cardiorespiratory arrest, heart attack and chest pains), arthritis or synovitis, arthralgia or myalgia and hypothrombinemia. Because of the small number of PT reported among some SOCs, a global analysis was performed for all PT reported among the three following SOCs: “psychiatric disorders”, “hepatobiliary disorders” and “renal and urinal disorders”. For “skin and subcutaneous tissue disorders” SOC,

we conducted two separated analyses, one on severe skin disorders: Stevens-Johnson syndrome (SJS) or toxic epidermal necrolysis (TEN) or acute generalized exanthematous pustulosis (AGEP); and one on all mild skin disorders (rash or erythema).

Non-cases were defined as reports of any other suspected ADR.

#### *5.2.3.3 Exposure definition*

Exposure was identified in the ICSR by the use of levamisole (Anatomical Therapeutic Chemical (ATC) code P02CE) preceding the onset of the adverse reaction.

#### *5.2.3.4 Statistical analysis*

Descriptive statistics were used to summarize the basic characteristics according to the indication of levamisole: anti-infectious, immunomodulator, adulterant or unknown/unprecise indication.

The indication categories were retrieved based on the information available in Vigibase® and defined as follows. The "anti-infectious" category includes cases where the drug was administered for any infection according to the market authorization (MA), *i.e.* for parasitic infections, or off-MA, *i.e.* at the discretion of the prescribing physician, for viral or bacterial infections. The "adulterant" category includes all cases where cocaine was part of the co-administered molecules or where the reporter notified that it was a misuse. The "immunomodulator" category includes all cases where levamisole is defined as an adjuvant, immunomodulator or anti-cancer treatment or where 5-fluorouracil was part of the co-administered drugs. Finally, the "unknown/unprecise" category includes all other cases where the reporter did not report any specific indication.

An analysis of characteristics associated with each ADR of interest where levamisole was suspected, including sex ratio, age, percentage of cases considered as serious, median time from initiation of levamisole to effect, reported use, reported period of notification and frequency of administration (single dose or multiple doses), was conducted. The reporting period was categorized into 4 categories (before 1990, between 1990 and 1999, between 2000 and 2009 and after 2009) according to important dates in the history of levamisole (approved in 1970 for its anthelmintic action; approved in 1990 for its immunomodulatory action; loss of marketing authorizations in the 2000s; first case of levamisole uses as a cocaine adulterant in 2009).

Our primary analyses consisted in calculating the ROR of each suspected ADR of interest (and corresponding 95% confidence interval [95% CI]) for levamisole compared to all other drugs reported in Vigibase® using logistic regression models. A first secondary stratified analysis consisted in calculating the ROR of each suspected ADR of interest for levamisole compared to mebendazole but only when the indication was anti-infectious. In two last secondary analyses, ADRs were compared according to levamisole type of use: immunomodulatory indication versus anti-infectious indication, and adulterant use versus anti-infectious indication.

Analyses were conducted using STATA v.15.1 software (StatCorps, LP, College Station, TX, USA).

#### *5.2.3.5 Description of the mechanisms of action of levamisole*

A separate literature review was performed using the Medline database to search information on the mechanisms of action of levamisole, their potential implication in occurrence of ADRs and their potential synergy with cocaine or 5-fluorouracil.

### 5.2.4 Results

#### *5.2.4.1 Descriptive analysis of the ADRs reported with levamisole*

Among the 24 217 750 cases reported in Vigibase® and after elimination of duplicates, 1763 suspected ADRs after administration of levamisole were reported between February 27, 1977 and February 7, 2021. Among them, 265 (15.0%) were reported as serious, 89 (5.0%) resulted in death, 82 (4.6%) involved cocaine, 142 (8.0%) occurred after use for an anti-infectious indication, 953 after use for immunomodulatory action (54.1%) and 586 (33.2%) had no specified indication. Within immunomodulatory cases, 51 (5.3%) concerned treatment of pediatric nephrotic syndrome and 902 (94.7%) concerned cancer treatment in association with 5-fluorouracil. Table 31 shows the distribution of cases according to levamisole use by age, gender, dosage regimen, seriousness, reporter type, geographical area, and reporting period.

| | Levamisole use | | | | |
|---|---|---|---|---|---|
| | Adulterant (n=82) | Anti-infectious (n=142) | Immunomodulator (n=953) | Unknown/Unprecise (n=586) | Total (n=1763) |
| **Age** | | | | | |
| < 18 years | 1 (1.4%) | 31 (23.3%) | 49 (6.0%) | 66 (13.4%) | 147 (9.7%) |
| 18-65 years | 69 (97.2%) | 96 (72.2%) | 449 (55.2%) | 353 (71.6%) | 967 (64.0%) |
| > 65 years | 1 (1.4%) | 6 (4.5%) | 315 (38.7%) | 74 (15.0%) | 396 (26.2%) |
| Missing data | 11 | 9 | 140 | 93 | 253 |
| **Sex** | | | | | |
| Female | 33 (42.9%) | 72 (51.0%) | 452 (51.9%) | 310 (58.0%) | 867 (53.4%) |
| Male | 44 (57.1%) | 69 (49.0%) | 419 (48.1%) | 224 (42.0%) | 756 (46.6%) |
| Missing data | 5 | 1 | 82 | 52 | 140 |
| **Dosage regimen** | | | | | |
| Single dose | 0 | 72 (60.0%) | 5 (1.5%) | 105 (37.1%) | 182 (25.0%) |
| Multiple doses | 0 | 48 (40.0%) | 319 (98.5%) | 178 (62.9%) | 545 (75.0%) |
| Missing data | 82 | 22 | 629 | 301 | 1036 |
| **Seriousness** | | | | | |
| Yes | 80 (97.6%) | 19 (21.3%) | 42 (70.0%) | 124 (65.3%) | 265 (62.9%) |
| No | 2 (2.4%) | 70 (78.7%) | 18 (30.0%) | 66 (34.7%) | 156 (37.1%) |
| Missing data | 0 | 53 | 893 | 396 | 1342 |
| **Seriousness criterion** | | | | | |
| Caused/prolonged hospitalization | 19 (23.7%) | 5 (38.5%) | 8 (20.0%) | 82 (66.7%) | 114 (44.5%) |
| Death | 55 (68.7%) | 0 | 3 (7.5%) | 31 (25.2%) | 89 (34.8%) |
| Disabling/incapacitating | 0 | 0 | 2 (5.0%) | 0 | 2 (0.8%) |
| Life threatening | 2 (2.5%) | 2 (15.4%) | 1 (2.5%) | 5 (4.1%) | 10 (3.9%) |
| Other important condition | 4 (5.0%) | 6 (46.1%) | 26 (65.0%) | 5 (4.1%) | 41 (16.0%) |
| **Reporter** | | | | | |
| Health professionals | 74 (96.1%) | 108 (99.0%) | 213 (100%) | 433 (98.9%) | 828 (98.9%) |
| Other occupations | 3 (3.9%) | 1 (1.0%) | 0 | 5 (1.1%) | 9 (1.1%) |
| Missing data | 5 | 33 | 740 | 148 | 926 |
| **Continent** | | | | | |
| Africa | 0 | 22 (15.5%) | 0 | 70 (11.9%) | 92 (5.2%) |
| North America | 55 (67.1%) | 1 (0.7%) | 794 (83.3%) | 147 (25.1%) | 997 (56.6%) |
| South America | 0 | 15 (10.6%) | 0 | 56 (9.6%) | 71 (4.0%) |
| Asia | 1 (1.2%) | 79 (55.6%) | 8 (0.8%) | 63 (10.7%) | 151 (8.6%) |
| Australia | 0 | 4 (2.8%) | 40 (4.2%) | 27 (4.6%) | 71 (4.0%) |
| Europa | 26 (31.7%) | 21 (14.8%) | 111 (11.7%) | 223 (38.0%) | 381 (21.6%) |
| **Reporting period** | | | | | |
| Before 1990 | 0 | 15 (10.6%) | 1 (0.1%) | 143 (24.4%) | 159 (9.0%) |
| 1990 – 1999 | 0 | 10 (7.0%) | 816 (85.6%) | 140 (23.9%) | 966 (54.8%) |
| 2000 - 2009 | 5 (6.1%) | 26 (18.3%) | 88 (9.2%) | 100 (17.1%) | 219 (12.4%) |
| After 2010 | 77 (93.9%) | 91 (64.1%) | 48 (5.0%) | 203 (34.6%) | 419 (23.8%) |

**Tableau 31. Characteristics of levamisole-related ADRs reported in VigiBase® according to the indication**

n, number of cases

Most cases concerned adults (64.0%) and were reported by healthcare professionals (98.9%). Mean age was 35.7 ± 23.9 years for all cases, 35.7 ± 12.7 years for adulterant cases, 23.4 ± 19.5 years for anti-infectious cases, 59.0 ± 16.7 years for immunomodulator cases, 9.0 ± 3.3 years for nephrotic syndrome and 61.9 ± 11.8 years for cancer therapy. Suspected ADRs were more frequently fatal in adulterant cases (55 deaths; 68.7%) than in other indications. All deaths that occurred in cases where levamisole was used as an adulterant also cited cocaine as a potential suspect. The four countries that reported the highest number of cases were the USA (958 cases, 54.2%), the United Kingdom (98, 5.5%), France (75, 4.2%) and India (75, 4.2%). Single dose was more frequent in anti-infectious cases than in immunomodulator or unknown indication cases. The three most reported SOC were “general disorders and administration site conditions” (25.8%), “nervous system disorders” (23.9%) and “skin and subcutaneous tissue disorders” (20.9%) (Table 32).

#### *5.2.4.2 Description of ADRs related to levamisole*

Description of adverse events where levamisole was suspected are presented in Table 33.

With the exception of convulsions, thrombocytopenia, neuropathy, severe skin disorders and failures, the majority of ADRs were more frequently reported in women than in men. The median time to onset of ADR was lower than 2 weeks for vasculitis, convulsions, thrombocytopenia, severe skin disorders, rashes, vertigo, fever, failures, arthralgia/myalgia and hypothrombinemia, and higher than 2 weeks for encephalopathy, agranulocytosis, neutropenia, tachycardia and arthritis/synovitis. Relatively few levamisole-related ADRs were reported for the anti-infectious indication and adulterant use, comprising less than 8% and 5% of reported cases, respectively. Similarly, relatively few ADRs were reported following a single dose of levamisole.

#### *5.2.4.3 Disproportionality analysis*

The results of the disproportionality analyses of levamisole-related ADRs of interest compared to any other drugs are presented in Table 34.

| **System Organ Class (SOC)**, n (% of ICSR with mention of the SOC) | **Adulterant** (n=82) | **Anti-infectious** (n=142) | **Immunomodulator** (n=953) | **Unknown/Unprecise** (n=586) | **Total** (n=1763) |
|---|---|---|---|---|---|
| General disorders and administration site conditions | 30 (37.0%) | 28 (19.7%) | 221 (23.2%) | 174 (29.7%) | 455 (25.8%) |
| Nervous system disorders | 8 (9.9%) | 36 (25.4%) | 246 (25.8%) | 132 (22.5%) | 422 (23.9%) |
| Skin and subcutaneous tissue disorders | 3 (3.7%) | 30 (21.2%) | 180 (18.9%) | 154 (26.3%) | 369 (20.9%) |
| Gastrointestinal disorders | 3 (3.7%) | 79 (55.6%) | 151 (15.8%) | 118 (20.1%) | 352 (19.9%) |
| Blood and lymphatic system disorders | 13 (16.0%) | 7 (4.9%) | 118 (12.4%) | 96 (16.4%) | 234 (13.3%) |
| Psychiatric disorders | 32 (39.5%) | 14 (9.9%) | 81 (8.5%) | 56 (9.6%) | 183 (10.4%) |
| Musculoskeletal, connectives tissues disorders | 6 (7.4%) | 5 (3.5%) | 98 (10.3%) | 62 (10.6%) | 171 (9.7%) |
| Investigations | 9 (11.1%) | 0 | 92 (9.7%) | 26 (4.4%) | 127 (7.2%) |
| Metabolism and nutrition disorders | 3 (3.7%) | 4 (2.8%) | 99 (10.4%) | 20 (3.4%) | 126 (7.1%) |
| Vascular disorders | 9 (11.1%) | 5 (3.5%) | 45 (4.7%) | 27 (4.6%) | 86 (4.9%) |
| Injury, poisoning, procedural complications | 57 (70.4%) | 0 | 8 (0.8%) | 20 (3.4%) | 85 (4.8%) |
| Respiratory, thoracic, mediastinal disorders | 12 (14.8%) | 4 (2.8%) | 34 (3.6%) | 28 (4.8%) | 78 (4.4%) |
| Infections and infestations | 5 (6.2%) | 1 (0.7%) | 53 (5.6%) | 16 (2.7%) | 75 (4.2%) |
| Hepatobiliary disorders | 0 | 0 | 59 (6.2%) | 13 (2.2%) | 72 (4.1%) |
| Renal and urinal disorders | 5 (6.2%) | 4 (2.8%) | 39 (4.1%) | 17 (2.9%) | 65 (3.7%) |
| Cardiac disorders | 15 (18.5%) | 1 (0.7%) | 26 (2.7%) | 21 (3.6%) | 63 (3.6%) |
| Eye disorders | 0 | 3 (2.1%) | 37 (3.9%) | 18 (3.1%) | 58 (3.3%) |
| Immune system disorders | 0 | 4 (2.8%) | 10 (1.0%) | 8 (1.4%) | 22 (1.2%) |
| Neoplasm benign, malignant and unspecified | 0 | 0 | 15 (1.6%) | 2 (0.3%) | 17 (1.0%) |
| Ear and labyrinth disorders | 0 | 2 (1.4%) | 6 (0.6%) | 8 (1.4%) | 16 (0.9%) |
| Endocrine disorders | 2 (2.5%) | | 9 (0.9%) | 1 (0.2%) | 12 (0.7%) |
| Reproductive system and breast disorders | 0 | 2 (1.4%) | 4 (0.4%) | 2 (0.3%) | 8 (0.5%) |
| Pregnancy, puerperium, perinatal disorders | 1 (1.2%) | 2 (1.4%) | 0 | 1 (0.2%) | 4 (0.2%) |
| Product issues | 2 (2.5%) | 0 | 0 | 1 (0.2%) | 3 (0.2%) |
| Congenital, familial and genetic disorders | 0 | 1 (0.7%) | 1 (0.1%) | 0 | 2 (0.1%) |
| Surgical and medical procedures | 1 (1.2%) | 0 | 0 | 1 (0.2%) | 2 (0.1%) |
| Social circumstances | 1 (1.2%) | 0 | 0 | 0 | 1 (0.1%) |

**Tableau 32. Levamisole-related ADRs: frequency of reported SOC by indications**

Multiple SOC can be reported in a single ICSR

| Adverse events | Sex Ratio [a] | Serious [b] | Time of occurrence [c] | Age (N, %) | | | Use (N, %) | | | | Notification period | | | | Dosage regimen | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | <18 years | 18-65 years | > 65 years | Adulterant | Anti-infectious | Immunomodulator | Unknown indication | Before 1990 | 1990 - 1999 | 2000 - 2009 | After 2010 | Single dose | Multiples doses |
| **Vasculitis** | 0.31 | 91.7% | 9 | **2** (11.8%) | **14** (82.4%) | **1** (5.9%) | **4** (23.5%) | **1** (5.9%) | **3** (17.5%) | **9** (52.9%) | **2** (12.5%) | **2** (12.5%) | **0** (0.0%) | **12** (75.0%) | **0** (0%) | **5** (29.4%) |
| **Encephalopathy** | 0.49 | 100% | 53 | **1** (1.7%) | **32** (54.2%) | **19** (32.2%) | **1** (1.7%) | **1** (1.7%) | **50** (84.8%) | **7** (11.9%) | **0** (0.0%) | **42** (71.2%) | **9** (15.2%) | **8** (13.6%) | **0** (0%) | **27** (45.8%) |
| **Neuropathy** | 1.30 | 100% | 47.5 | **0** (0%) | **10** (40.0%) | **7** (28.0%) | **1** (4.0%) | **0** (0%) | **21** (84.0%) | **3** (12.0%) | **2** (8.0%) | **15** (60.0%) | **6** (24.0%) | **2** (8.0%) | **0** (0%) | **8** (32.0%) |
| **Convulsions** | 1.37 | 50.0% | 8 | **1** (4.5%) | **10** (45.5%) | **6** (27.3%) | **0** (0%) | **1** (4.5%) | **13** (59.1%) | **8** (36.4%) | **1** (4.5%) | **19** (86.4%) | **0** (0%) | **2** (9.1%) | **1** (4.5%) | **12** (54.5%) |
| **Agranulocytosis** | 0.39 | 100% | 38 | **3** (5.0%) | **35** (58.3%) | **14** (23.3%) | **4** (6.7%) | **3** (5.0%) | **18** (30.0%) | **35** (58.3%) | **21** (35.0%) | **24** (40.0%) | **6** (10.0%) | **9** (15.0%) | **0** (0%) | **28** (53.3%) |
| **Leucopenia** | 0.72 | 66.7% | 19 | **3** (4.3%) | **43** (61.4%) | **20** (28.6%) | **1** (1.4%) | **1** (1.4%) | **47** (67.1%) | **21** (30.0%) | **15** (21.4%) | **46** (65.7%) | **6** (8.6%) | **3** (4.3%) | **0** (0%) | **31** (44.3%) |
| **Neutropenia** | 0.74 | 73.7% | 26 | **16** (28.6%) | **24** (42.9%) | **13** (23.2%) | **7** (12.5%) | **2** (3.6%) | **17** (30.4%) | **30** (53.6%) | **21** (37.5%) | **5** (8.9%) | **11** (19.6%) | **19** (33.9%) | **0** (0%) | **32** (57.1%) |
| **Thrombocytopenia** | 1.00 | 75.0% | 9 | **3** (9.4%) | **15** (47.0%) | **11** (34.4%) | **2** (6.3%) | **1** (3.1%) | **22** (68.8%) | **7** (21.9%) | **5** (15.6%) | **20** (62.5%) | **3** (9.4%) | **4** (12.5%) | **0** (0%) | **14** (43.8%) |
| **SJS/ TEN/ APEG** | 1.50 | 100% | 9.5 | **1** (20.0%) | **4** (80.0%) | **0** (0%) | **0** (0%) | **0** (0%) | **3** (60.0%) | **2** (40.0%) | **0** (0%) | **3** (60.0%) | **0** (0%) | **2** (40.0%) | **0** (0%) | **1** (20.0%) |
| **Rashes** | 0.65 | 33.3% | 11 | **21** (11.2%) | **95** (50.8%) | **51** (27.3%) | **0** (0%) | **12** (6.4%) | **109** (58.3%) | **67** (35.6%) | **28** (15.0%) | **124** (66.3%) | **14** (7.5%) | **21** (11.2%) | **12** (6.4%) | **67** (35.8%) |
| **Vertigo** | 0.80 | 58.3% | 1 | **5** (6.7%) | **54** (72.0%) | **11** (14.7%) | **0** (0%) | **16** (21.3%) | **25** (33.3%) | **34** (45.3%) | **3** (4.0%) | **28** (37.3%) | **15** (20.0%) | **29** (38.7%) | **27** (36.0%) | **20** (26.7%) |
| **Fever** | 0.65 | 68.4% | 9 | **11** (8.3%) | **84** (63.4%) | **29** (22.0%) | **5** (3.8%) | **14** (10.6%) | **54** (40.9%) | **59** (44.7%) | **34** (25.8%) | **60** (45.5%) | **20** (15.1%) | **18** (13.6%) | **15** (11.4%) | **49** (37.1%) |
| **Tachycardia** | 0.36 | 100% | 35 | **1** (6.3%) | **13** (81.2%) | **1** (6.3%) | **1** (6.2%) | **0** (0%) | **9** (56.2%) | **6** (37.5%) | **2** (12.5%) | **10** (62.5%) | **1** (6.2%) | **3** (18.8%) | **0** (0%) | **5** (31.3%) |
| **Failures** | 1.16 | 95.5% | 2 | **0** (0%) | **34** (80.9%) | **5** (11.9%) | **10** (23.8%) | **0** (0%) | **16** (38.1%) | **16** (38.1%) | **0** (0%) | **17** (40.5%) | **3** (7.1%) | **22** (52.4%) | **9** (21.4%) | **11** (26.2%) |
| **Arthritis/Synovitis** | 0.75 | M.D. | 30 | **1** (6.3%) | **7** (43.7%) | **4** (25.0%) | **0** (0%) | **0** (0%) | **12** (66.7%) | **6** (33.3%) | **1** (6.2%) | **12** (75.0%) | **3** (18.8%) | **0** (0%) | **0** (0%) | **6** (37.5%) |
| **Arthralgia/Myalgia** | 0.74 | 86.0% | 2 | **7** (7.0%) | **68** (68.0%) | **15** (15.0%) | **3** (3.0%) | **4** (4.0%) | **57** (57.0%) | **36** (36.0%) | **12** (12.0%) | **57** (57.0%) | **11** (11.0%) | **20** (20.0%) | **17** (17.0%) | **25** (25.0%) |
| **Hypothrombinemia** | 0.78 | M.D. | 16.5 | **0** (0%) | **11** (35.5%) | **13** (41.9%) | **0** (0%) | **0** (0%) | **27** (87.1%) | **4** (12.9%) | **0** (0%) | **31** (100%) | **0** (0%) | **0** (0%) | **0** (0%) | **6** (19.4%) |

**Tableau 33. Description of ADRs related to levamisole**

[a] Ratio Male/Female

[b] Percentage of cases reported as serious

[c] Median time to onset of effect from initiation of treatment (in days)

M.D., missing data

For age, indication, reporting period and dosage regimen, the lines contain the number of cases and the percentage over all cases. Missing data are not described but are included in the percentage calculations

| System Organ Class | Preferred Term | Primary analysis [a] | | | Secondary analysis 1 [b] | | | Secondary analysis 2 [c] | | | Secondary analysis 3 [d] | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | N | ROR (95% CI) | *P* | N | ROR (95% CI) | *P* | N | ROR (95% CI) | *P* | N | ROR (95% CI) | *P* |
| Psychiatric disorders | All PT | 183 | 1.4 (1.2–2.6) | 0.050 | 13 | 4.0 (2.0–7.8) | < 0.001 | 72 | 0.8 (0.4–1.5) | 0.509 | 32 | 7.4 (3.5–15.3) | < 0.001 |
| Hepatobiliary disorders | All PT | 72 | 2.4 (1.9–4.3) | 0.009 | 0 | No cases* | . | 58 | No cases* | . | 0 | No cases* | |
| Renal and urinal disorders | All PT | 65 | 1.3 (1.0–2.3) | 0.065 | 3 | 1.4 (0.4–4.7) | 0.608 | 42 | 2.1 (0.6–6.9) | 0.209 | 5 | 3.3 (0.8–14.2) | 0.108 |
| Vascular disorders | Vasculitis | 17 | 6.5 (4.1–10.6) | <0.001 | 1 | 8.3 (0.5–133) | 0.136 | 3 | 0.4 (0.1–4.3) | 0.485 | 4 | 7.9 (0.9–72.4) | 0.066 |
| Nervous system disorders | Encephalopathy | 59 | 22.5 (17.4–39.9) | <0.001 | 1 | No cases** | . | 50 | 7.8 (1.1–57.0) | 0.043 | 1 | 1.9 (0.1–30.9) | 0.650 |
| Nervous system disorders | Neuropathy | 25 | 4.3 (2.9–7.1) | <0.001 | 0 | No cases* | . | 21 | No cases* | . | 1 | No cases* | . |
| Nervous system disorders | Convulsions | 22 | 1.4 (0.9–2.4) | 0.064 | 1 | 0.4 (0.1–3.4) | 0.444 | 13 | 1.9 (0.2–15.0) | 0.521 | 0 | No cases*** | . |
| Blood and lymphatic system disorders | Agranulocytosis | 60 | 25.2 (19.5–44.7) | <0.001 | 3 | No cases** | . | 18 | 0.9 (0.2–3.1) | 0.857 | 4 | 2.6 (0.6–12.0) | 0.217 |
| Blood and lymphatic system disorders | Leucopenia | 70 | 9.8 (7.7–17.5) | <0.001 | 1 | 4.1 (0.4–46.0) | 0.247 | 47 | 7.3 (1.0–53.5) | 0.050 | 1 | 1.9 (0.1–30.9) | 0.650 |
| Blood and lymphatic system disorders | Neutropenia | 56 | 4.8 (3.7–8.5) | <0.001 | 2 | 3.3 (0.6–17.3) | 0.153 | 17 | 1.3 (0.3–5.6) | 0.749 | 7 | 7.2 (1.4–35.6) | 0.015 |
| Blood and lymphatic system disorders | Thrombocytopenia | 32 | 2.8 (2.0–4.8) | <0.001 | 1 | 4.1 (0.4–46.0) | 0.247 | 22 | 3.3 (0.4–24.9) | 0.241 | 2 | 3.9 (0.3–43.3) | 0.273 |
| Skin and subcutaneous tissue disorders | SJS/TEN/AGEP [e] | 5 | 1.3 (0.6–1.9) | 0.093 | 0 | No cases* | . | 3 | No cases* | . | 0 | No cases* | . |
| Skin and subcutaneous tissue disorders | All rashes [f] | 187 | 1.5 (1.3–2.8) | 0.042 | 12 | 0.7 (0.4-1.4) | 0.337 | 109 | 1.4 (0.7–2.6) | 0.290 | 0 | No cases*** | . |
| General disorders | Vertigo | 75 | 1.1 (0.8–1.9) | 0.088 | 16 | 1.8 (1.0–3.2) | 0.038 | 25 | 0.2 (0.1–0.4) | < 0.001 | 0 | No cases*** | . |
| General disorders | Fever | 132 | 2.4 (2.0–4.4) | 0.008 | 14 | 2.7 (1.5–5.1) | <0.001 | 54 | 0.5 (0.3–1.0) | 0.057 | 5 | 0.65 (0.2–1.9) | 0.432 |
| Cardiac disorders | Tachycardia | 16 | 1.3 (0.8–2.2) | 0.074 | 0 | No cases* | . | 9 | No cases* | . | 1 | No cases* | . |
| Cardiac disorders | Failures [g] | 42 | 1.0 (0.7–1.7) | 0.101 | 0 | No cases* | . | 16 | No cases* | . | 10 | No cases* | . |
| Musculoskeletal disorders | Arthritis/Synovitis | 18 | 3.9 (2.4–6.3) | < 0.001 | 0 | No cases* | . | 12 | No cases* | . | 0 | No cases* | . |
| Musculoskeletal disorders | Arthralgia/Myalgia | 100 | 2.3 (1.9–4.2) | 0.010 | 4 | 4.2 (1.2–14.2) | 0.020 | 57 | 2.2 (0.8–6.1) | 0.134 | 3 | 1.4 (0.3–6.6) | 0.641 |
| Investigations | Hypothrombinemia | 31 | 41.5 (29.1–70.6) | < 0.001 | 0 | No cases* | | 27 | No cases* | . | 0 | No cases* | . |

**Tableau 34. Disproportionality analysis of levamisole-related adverse reactions at Preferred Term level**

N: number of exposed cases, ROR: reporting odds ratio, CI: confidence interval

* No cases when levamisole is used as an anti-infective; ** No cases in mebendazole group; *** No cases when levamisole is used as an adulterant

[a] Primary analysis comparing levamisole-related cases with all cases reported in the WHO' pharmacovigilance database

[b] Secondary analysis comparing ADRs after use of levamisole for an anti-infective indication with those occurring after use of mebendazole (control group)

[c] Secondary analysis comparing ADRs after use of levamisole for an immunomodulatory indication with those after its use for an anti-infective indication (control group)

[d] Secondary analysis comparing ADRs after use of levamisole for an adulterant action with those after its use for an anti-infective indication (control group)

[e] Stevens-Johnson syndrome or Toxic epidermal necrolysis or Acute generalized exanthematous pustulosis

[f] Regroups all rashes and all erythema

[g] Regroups cardiac arrest, cardiorespiratory arrest, heart attack and chest pains

The relative frequencies of psychiatric disorders, hepatobiliary disorders, encephalopathies, neuropathy, agranulocytosis, leucopenia, neutropenia, thrombocytopenia, fever, arthritis/synovitis, arthralgia/myalgia, hypothrombinemia and vasculitis were significantly higher with levamisole than with other drugs (see all ROR values and 95% CI in Table 34). Psychiatric disorders were significantly more frequently reported after levamisole-adulterated cocaine intake than after levamisole intake for anti-infectious indication. No cases of hepatobiliary disorders, encephalopathy, neuropathy, agranulocytosis, serious skin disorders (SJS, TEN and AGEP), arthritis/synovitis, tachycardia, failures or hypothrombinemia were reported when levamisole was given for an anti-infectious indication. Encephalopathies and leucopenia were more frequently reported when levamisole was used for an immunomodulatory action than when it was used for an anti-infectious indication. Neutropenia was more frequently reported when levamisole was misused than when it was used for its anti-infectious action. The association between vasculitis and levamisole intake disappeared in the three secondary analyses. Arthralgia/myalgia were also more frequently reported with levamisole compared to mebendazole for anti-infectious purposes. Serious skin and subcutaneous tissue disorders were not more frequently reported with levamisole than with other drugs. Vertigo was more frequently reported with levamisole than with mebendazole and when levamisole was used for an anti-infectious purpose than for an immunomodulatory purpose.

#### *5.2.4.4 Levamisole mechanisms of action*

Table 35 summarizes the mechanisms of action of levamisole identified from the scientific literature, their potential implication in the occurrence of ADRs and their potential synergy with cocaine or 5-fluorouracil. We identified 11 different pharmacological mechanisms for levamisole. For each identified mechanism, we summarized the pharmacological effects both on worms and humans.

| Mechanisms | Potential effects | | Potential synergy | References |
|---|---|---|---|---|
| | On worms | On humans | | |
| Nicotinic receptor agonist and allosteric modulator | Reduces the capacity of male worms to control their reproductive muscles and limits their ability to copulate | Mimics the effects of acetylcholine on nicotine receptors | Increases the pleasurable and behavior reinforcing effects of cocaine | (Raymon and Isenschmid 2009; Levandoski et al. 2003) |
| Inhibition of cyclic AMP-mediated glycogenolysis | Increases glucose incorporation into glycogen and decrease glycogen phosphorylase activity ratios | | | (Harris 1986) |
| Selective inhibition of MAO-A and COMT | | Resembles certain antidepressant drugs<br>Limits the degradation of dopamine<br>Increases dopamine concentration in the cerebral reward pathway | Potentiates the dopamine level due to cocaine inhibitory action in dopamine reuptake | (Vanhoutte et al. 1977; Shah et al. 1986; Spector et al. 1998) |
| Decrease of norepinephrine reuptake | | Resembles certain antidepressant drugs<br>Convulsions at high doses | Potentiates the norepinephrine release due to cocaine at sympathetic synapsis level | (Van Nueten 1972; Vanhoutte et al. 1977; Pires et al. 1979) |
| Anticholinesterase activity | Increases the concentration of acetylcholine | Increases the concentration of acetylcholine | Increases the pleasurable and behavior reinforcing effects of cocaine | (Shah et al. 1986; Pires et al. 1979) |
| Endogenous opioid synthesis | | Increases endogenous opioid concentrations in specific areas of the brain and in peripheral tissues | Potentiates cocaine effects | (Spector et al. 1998) |
| Metabolization into an amphetamine-like compound (aminorex) | | Modulates norepinephrine, dopamine and serotonin levels | Potentiates cocaine effects | (Ho et al. 2009; Barker 2009; Bertol et al. 2011; Hofmaier et al. 2014) |
| Local anesthetic properties | | | | (Onuaguluchi and Igbo 1987) |
| Stimulation of T-cell | | Activates and induces proliferation of T-cells | Potentiates 5-fluorouracil immunomodulatory activities | (Renoux 1980; Abdalla et al. 1995) |
| Potentiation of monocyte and macrophage functions | | Increases phagocytosis and chemotaxis | | |
| Increase neutrophil functions | | Increases mobility, adherence and chemotaxis | | |

**Tableau 35. Mechanisms of action of levamisole and their potential synergy of action with 5-fluorouracil and cocaine.**

MAO-A: monoamine oxidase type A, COMT: catechol-omethyl transferase

### 5.2.5 Discussion

Our study used a case-non-case approach to analyze data collected in the WHO drug adverse events database from 1977 to 2021 to assess the association between levamisole use and the reporting of suspected ADRs of interest. To our knowledge, it is the first study to review the main ADRs associated with levamisole. Significant disproportionality signals were found, with our results showing more frequent reporting of psychiatric disorders, hepatobiliary disorders, vasculitis, encephalopathies, neuropathies, agranulocytosis, leucopenia, neutropenia, thrombocytopenia, mild rashes, fever, arthritis, arthralgia and hypothrombinemia. When comparing levamisole to mebendazole in anti-infectious indications, we identified new pharmacovigilance signals regarding hepatobiliary disorders, neuropathy, serious skin disorders, tachycardia, failures, arthritis and hypothrombinemia. In addition, some other known ADR were not retrieved: leucopenia, neutropenia, thrombocytopenia, rashes and hypothrombinemia. One of our main hypotheses is that levamisole is used at single dose in the vast majority of anti-infectious indications and that this administration regimen results in far fewer ADRs, with this reduction likely most significant for serious ADRs. This hypothesis is supported by two secondary analyses. Encephalopathies and leucopenia were more frequently reported when levamisole was used for an immunomodulatory action compared to when it was used for an anti-infectious action, and psychiatric disorders and neutropenia were more frequently reported when it was used as an adulterant than for its anti-infectious activity.

The majority of the levamisole-related ADRs concerned either its use in immunomodulatory indications, or when delivered as a multiple dose regimen. The median times to onset of each ADR suggest that the drug induces short-term effects (vasculitis, convulsions, thrombocytopenia, rashes, vertigo, fever, failures, arthralgia, hypothrombinemia) as well as delayed effects (encephalopathy, neuropathy, agranulocytosis, leucopenia, neutropenia, tachycardia and arthritis). These delayed effects could be immuno-mediated effects, potentially induced by the immunomodulatory properties of levamisole. If clinical trials on the use of levamisole in patients with COVID-19 give good results, its benefit-risk balance as an immunomodulator in this infection will have to be re-evaluated to enable its use in hospital or

ambulatory settings. In June 2021, four clinical trials evaluating levamisole in the management of COVID-19 were reported in ClinicalTrials.gov, and the results of one of them have been published. The authors conclude that levamisole could potentially improve the cough and dyspnea of patients with COVID-19 but no benefit could be demonstrated on mortality or aggravation of the disease (Roostaei Firozabad et al. 2021).

The mechanisms of action of levamisole are multiple. It acts at the level of nicotinic receptors (Raymon and Isenschmid 2009; Levandoski et al. 2003), the glucose pathway (Harris 1986), dopaminergic pathways (Vanhoutte et al. 1977; Shah et al. 1986; Spector et al. 1998), norepinephrine and acetylcholine (Van Nueten 1972; Vanhoutte et al. 1977; Pires et al. 1979; Shah et al. 1986). In addition, secondary mechanisms exist such as its capacity to increase endogenous opiate synthesis (Spector et al. 1998), to metabolize into an amphetamine-like compound (Ho et al. 2009; Barker 2009; Bertol et al. 2011), and to act on the immune system (Renoux 1980; Abdalla et al. 1995). Some of its mechanisms of action are synergistic with those of 5-fluorouracil, used in the treatment of cancers, or with those of cocaine. The fact that levamisole is often administered in combination with cocaine or 5-fluorouracil and the potential synergy of action between these molecules make it difficult to differentiate the molecule most likely to cause certain adverse effects.

Our study has several strengths. First, we used the global ADRs database VigiBase® intended to collect information on suspected ADRs from nearly all national pharmacovigilance systems in the world, allowing us to identify new pharmacovigilance signals for rare events with sufficient statistical power and to stratify on levamisole indication in secondary analyses. Second, our results are consistent with already known risks associated with levamisole (encephalopathy, agranulocytosis and vasculitis). Third, the analysis of real-life surveillance data with disproportionality analyses have already demonstrated their usefulness for detecting drug risks (Maciá-Martínez et al. 2016; Montastruc et al. 2011).

One of the main limitations of this study, inherent to all studies using pharmacovigilance databases (Bégaud et al. 2002; Van Der Heijden et al. 2002), is related to the potential missing information. Under-reporting of suspected ADRs, differences in the capacity of reporting between countries and the lack of information about the total number of patients exposed to

the drug may cause biased estimates. Nevertheless, there is no apparent reason that, in a specific region, ADRs would be more or less reported with levamisole than those occurring after treatment with any other drugs. Whilst this might mitigate potential bias in the results presented here, these results should be still interpreted with caution because of this potential missing information. Additionally, pharmacovigilance systems are not yet well established in African countries. In 2017, only 30% of these countries had specific procedures for the monitoring of ADRs and only 28% had a national platform for coordinating pharmacovigilance activities (Kaboré et al. 2017). Despite the widespread usage of levamisole in some African, Latin American or Asian countries, there remains little information about its use or potential ADRs arising from this use. However, our analyses suggest a good safety profile of single-dose levamisole for anthelmintic treatment and its use could be considered in some focal areas where emergence of benzimidazole resistance may occur, due to the high drug pressure caused by mass administration of albendazole or mebendazole (Zuccherato et al. 2018).

### 5.2.6 Conclusion

**Dans cet article, nous avons étudié tous les effets indésirables signalés après une prise de lévamisole à partir de la base de données de pharmacovigilance de l'OMS. Nous avons comparé les notifications des effets indésirables en fonction du type d'utilisation et du régime d'administration du lévamisole. Nos résultats indiquent que les effets indésirables liés au lévamisole diffèrent notablement selon l'indication pour laquelle il est utilisé.**

**La majorité des effets indésirables rapportés comme liés au lévamisole sont survenus lorsque le médicament a été administré pour une indication immunomodulatrice ou en mésusage, signant un schéma d'administration à doses élevées et/ou multiples.**

**Cette analyse systématique des effets indésirables rapportés comme liés au lévamisole confirme que les traitements à dose unique pour une action anthelminthique ont un bon profil de sécurité.**

## 5.3 Connaissances sur son action sur les nématodes

Comme dit précédemment, le lévamisole était initialement prescrit pour son action antihelminthique, notamment sur les géohelminthes. En 2017, une méta-analyse portant sur la comparaison de l'efficacité de doses uniques à la posologie recommandée des principaux médicaments utilisés dans la lutte contre les géohelminthes (albendazole 400 mg, mébendazole 500 mg, lévamisole 80 mg ou 2.5 mg/kg, and pamoate de pyrantel 10 mg/kg) est parue. Elle définit le lévamisole comme ayant un taux de guérison de 97,3% (CI95% :84,2–99,6%), 10.3% (CI95% :2.4–35.2%) et 29,5% (CI95% :6,1–72,9%), respectivement sur l'ascaridiose, l'ankylostomose et la trichocéphalose. Concernant le taux de réduction des œufs présents dans les selles, le lévamisole montre une efficacité de 96,4% (CI95% : 82,3–100,0%), 61,8% (CI95% : 30,3–93,3%) et 28,3% (CI95% : 6,7–49,8%), respectivement sur l'ascaridiose, l'ankylostomose et la trichocéphalose. Ils concluent que le lévamisole montre une efficacité similaire aux autres antihelminthiques concernant l'ascaridiose et la trichocéphalose mais une efficacité inférieure concernant l'ankylostomose (Moser et al. 2017).

Concernant les filaires, nous trouvons des études portant sur l'onchocercose montrant une efficacité (définie comme la réduction de la microfiladermie post-traitement) de 30 et 44% après un traitement par dose unique de 2.5 mg/kg (Rivas-Alcalá et al. 1981; Awadzi et al. 2004) et des études concernant la filariose lymphatique (*W. bancrofti* ou *B. malayi*) montrant des taux de réduction de la microfilarémie variant de 78,8 à 98,5% (Merlin et al. 1976; McMahon 1979; Narasimham et al. 1978; O'Holohan and Zaman 1974; Zaman and Lal 1973). Le lévamisole n'a jamais été testé sur la loase.

La revue de ces différentes études suggère l'existence d'une relation effet-dose mais pas de relation effet-durée de traitement. L'efficacité du lévamisole est cependant bien inférieure à l'efficacité de l'ivermectine. Cette différence d'efficacité laisse imaginer la possibilité de l'utiliser comme traitement pour la loase, permettant de réduire progressivement, et donc de manière sûre, la densité microfilarienne de *L. loa* en dessous du seuil où les effets secondaires graves peuvent apparaitre.

Afin d'étudier cette hypothèse de recherche, nous avons réalisé un essai clinique randomisé contrôlé portant sur l'évaluation de la tolérance et de l'efficacité du lévamisole chez des individus porteurs de microfilaires de *L. loa* dans la circulation sanguine.

## 5.4 Efficacité du lévamisole sur la microfilarémie à *L. loa*

### Safety and efficacy of levamisole in loiasis: a randomized, placebo-controlled, double-blind clinical trial with ascending-dose and ascending-parasite density

*Clinical Infectious Diseases*

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**Running title**: Efficacy of levamisole on *Loa loa*

### 5.4.1 Abstract

**Background.** Individuals with high microfilarial densities (MFD) of *Loa loa* are at risk of developing serious adverse events (SAEs) after ivermectin treatment. Pretreatment with drugs progressively reducing *Loa* MFD below the risk threshold might help prevent these SAEs. We assessed the safety and efficacy of using levamisole for this purpose.

**Methods.** A double-blind, randomized, placebo-controlled, dose- and MFD-ascending trial was conducted in the Republic of the Congo. Participants were randomly assigned to receive single oral doses of levamisole (1.0, 1.5 or 2.5 mg/kg) or placebo. Safety outcomes were occurrence of SAE and frequency of adverse reactions during the first week. The efficacy outcomes were decrease of MFD, and proportions of individuals with at least 40% and 80% MFD reduction at day 2 (D2), D7 and D30.

**Results.** The two lowest doses (1.0 and 1.5 mg/kg) were safe (no SAE) but ineffective against *L. loa.* Mild AEs were more frequent after 2.5 mg/kg levamisole (10/85) than placebo (3/85) (P = .018). Median MFD reduction was higher after 2.5 mg/kg levamisole than placebo between baseline and D2 (-12.9% vs. + 15.5%, P < .001), D7 (-4.9% vs. +18.7%, P < .001) and D30 (-0.5% vs. +13.5%, P = .036). At D2, 17.5% of participants in the 2.5 mg/kg levamisole arm had a >40% reduction in their MFD (vs. 1.2% in placebo arm, P < .001).

**Conclusions.** Single-dose 2.5 mg/kg levamisole induces a transient reduction in *Loa* MFD. This promising result should encourage testing higher doses or longer regimens.

### 5.4.2 Introduction

Loiasis is a parasitic infection caused by the filarial nematode *Loa loa*. About 140 million people live in Central African regions where this disease is endemic (Zouré et al. 2011). Currently considered as benign by the World Health Organization (WHO), loiasis is a major obstacle to the elimination of onchocerciasis, another filarial disease. Since the 1990s, onchocerciasis control is based on mass treatment with ivermectin (IVM) of all meso- and hyperendemic communities. This has led to the local elimination of onchocerciasis in Latin America (WHO 2015; Sauerbrey et al. 2018) and some African foci (Diawara et al. 2009; Traore et al. 2012; Tekle et al. 2016), but not in Central Africa. The reason for this is that individuals with high densities of *Loa* microfilariae

(mfs) in the blood can develop a potentially fatal encephalopathy after IVM treatment (Gardon, Gardon-Wendel, Demanga-Ngangue, et al. 1997). These serious adverse events (SAEs) are probably due to the IVM-induced rapid paralysis of large numbers of *Loa* mfs, which leads to their passive drainage in the circulation and their embolization in brain capillaries. The current WHO goals are to "eliminate the transmission of onchocerciasis in 10 countries; to cease mass drug administration (MDA) with IVM in at least one focus in 34 countries; and to obviate the need for MDA in at least 25%, 50%, 75% and 100% of the population in at least 16, 14, 12, and 10 countries, respectively" by 2030 (Basáñez et al. 2019). However, this objective is jeopardized by the fact that some areas where onchocerciasis is hypoendemic are also endemic for loiasis; in these settings, the benefit-risk balance of implementing mass IVM treatment is unfavorable.

Alternative treatment strategies have been developed to safely combat onchocerciasis in hypoendemic areas which are co-endemic with loiasis. A solution to prevent post-IVM *Loa*-related SAEs would be to first treat the population with a drug to progressively reduce the *Loa* microfilarial density (MFD) below the threshold (30 000 mfs/mL) above which there is a risk of neurological SAE. Various drugs and regimens have already been tested for their suitability in this application such as albendazole (Klion et al. 1993; Kamgno and Boussinesq 2002; Tsague-Dongmo et al. 2002; Kamgno et al. 2016), antimalarials (Kamgno et al. 2010) or low doses of IVM (Kamgno J, Gardon J 2000; Kamgno J, Pion SD, Tejiokem MC, Twum-Danso NA, Thylefors B 2007) but none of these trials was successful: the effect was either too strong or too weak, or showed unsuitably large inter-individual variation.

Levamisole (LEV) is an long-established drug included in the WHO's List of Essential Medicines (World Health Organization 2019) and widely used in some countries, at a dose of 150 mg or 2.5 mg/kg, for its activity against soil-transmitted helminths (*Ascaris*, hookworms) (Moser et al. 2017). LEV had been tested in the early 1980s against *Onchocerca volvulus*, *Wuchereria bancrofti* and *Brugia malayi*. LEV showed moderate short-lasting activity in most trials (Miller 1980; O'Holohan and Zaman 1974; Zaman and Lal 1973; Rivas-Alcalá et al. 1981; Narasimham et al. 1978; Merlin et al. 1976; McMahon 1979; Awadzi et al. 1982; Moreau et al. 1975) but has never been tested against *Loa*. A synthesis of previous trials on the other filarial species is provided in Figures 22 and 23.

We report results of the first trial conducted to evaluate the safety and efficacy of single dose LEV in subjects infected with *Loa,* carried out in the Republic of the Congo.

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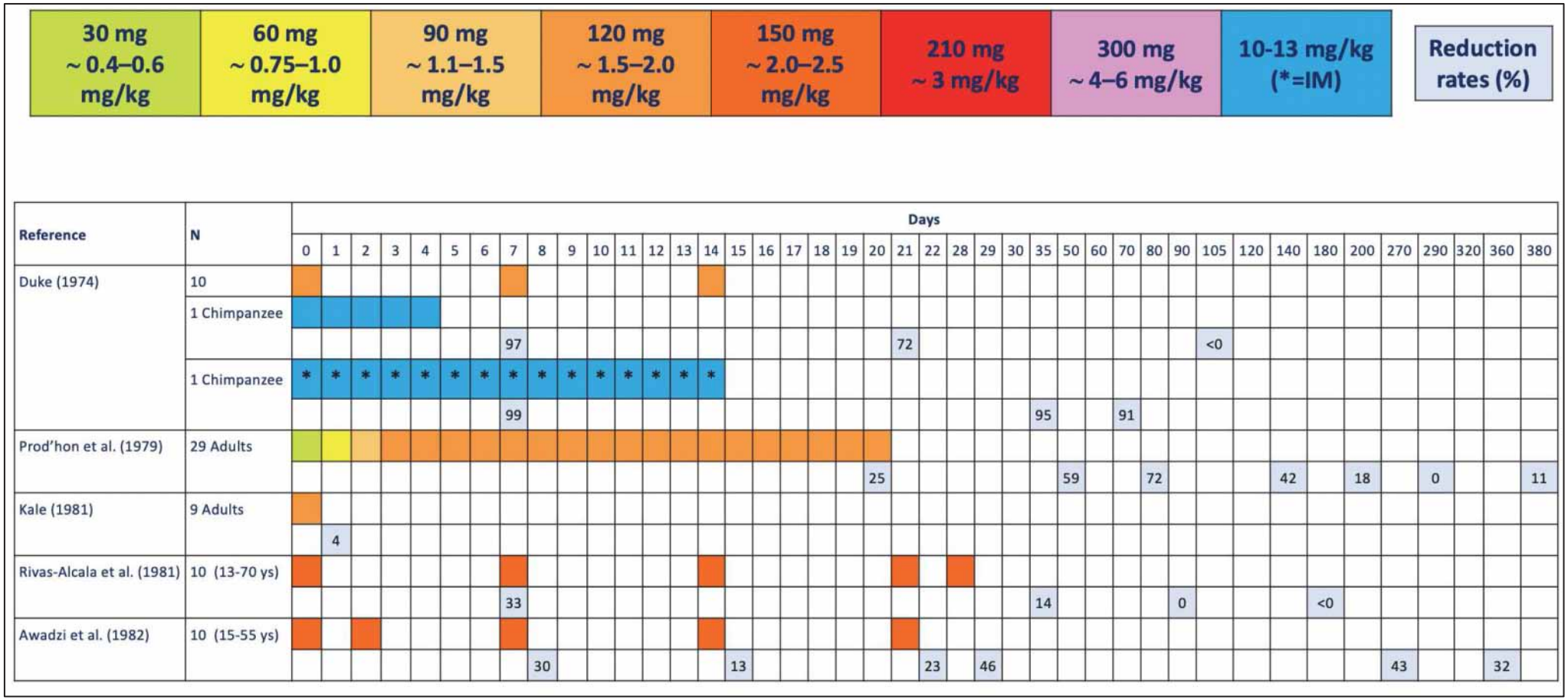


**Figure 22. Summary of the different studies on the efficacy of levamisole alone on Onchocerca volvulus**

*When levamisole is administered on the same day as a parasitological checkpoint, the study is summarized in 2 lines (he first for the treatment regimen used and the second for the microfilaremia reduction rates). If this is not the case, the study is summarized in one line.*

| 30 mg ~ 0.4–0.6 mg/kg | 60 mg ~ 0.75–1.0 mg/kg | 90 mg ~ 1.1–1.5 mg/kg | 120 mg ~ 1.5–2.0 mg/kg | 150 mg ~ 2.0–2.5 mg/kg | 210 mg ~ 3 mg/kg | 300 mg ~ 4–6 mg/kg |
|---|---|---|---|---|---|---|

Reduction rates (%)

| Reference | N (parasite) | Mean MFD [range] | Days | | | | | | | | | | | | | | | | | | | | | | | | | | | | | MFD at discharge/MFD at D0 (%) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | 0 | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 | 16 | 17 | 18 | 19 | 20 | 21 | 30 | 45 | 60 | 90 | 105 | 120 | 140 | 180 | |
| Zaman & Lal (1973) | 1 (Wb) | 247/20µL | | 97 | 94 | 90 | 73 | | | | | | | | | | | | | | | | | | | | | | | | | | |
| | 1 (Wb) | 10/20µL | | 100 | 80 | | | | 100 | | | | | | | | | | | | | | | | | | | | | | | | |
| O'Holohan & Zaman (1974) | 10 (Bm) | 5.9 [1-17]/20µL | | 75 | 85 | 52 | 47 | 34 | 71 | | | | | | | | | | | | | | | | | | | | | | | | |
| | 17 (Bm) | 8.2 [1-30]/20µL | | | | 94 | 91 | 85 | 91 | 91 | 87 | | | | | | | | | | | | | | | | | | | | | | |
| Moreau et al. (1975) | 27 (Wb) | 59.1 [2-453]/20µL | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| | | | | 53 | 72 | 91 | 91 | 94 | 96 | 95 | 96 | 99 | 99 | 98 | 99 | | | | | | | | | | | | 94 | | | | | | |
| Merlin et al. (1976) | 9 (Wb) | 12.4 [1-46.5]/20µL | | | | | | | | 93 | | | | | | | | | 90 | | | | | | 93 | | 88 | 84 | | | | | |
| | 1 (Wb) | 69/20µL | | | | | | | | 66 | | | | | | | | | 16 | | | | | | 16 | | 8 | <0 | | | | | |
| Narasimham et al. (1978) | 25 (Wb) | 52.5/20µL | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| | | | | | 66 | | 94 | | 97 | | 96 | | | | | 92 | | | | | 92 | | | | 95 | | 93 | 88 | | 87 | 87 | 88 | |
| McMahon (1979) | 1 (Wb) | 302/mL | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| | | | | | | | | | 96 | | | | | | | | | | | | | | | | 100 | | | | | | | | |
| | 1 (Wb) | 171/mL | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| | | | | | | | | | 95 | | | | | | | | | | | | | | | | 94 | | | | | | | | |
| | 1 (Wb) | 6572/mL | | | | | | | 99 | | | | | | | | | | | | | | | | 94 | 89 | 60 | 69 | | | | | |
| | 1 (Wb) | 3418/mL | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| | | | | | | | | | 17 | | | | | | | | | | | | | | | | 70 | | | 45 | | | | | |
| Perez Avila et al. (1980) | 5 (Wb) | 451.2 [95–1110]/mL | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| | | | | 95 | 98 | 100 | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Mak & Zaman (1980) | 10 (Wb/Bm) | 47.8 [5–233]/60µL | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | 1.7–240% (after 2–10 wks) |
| | 10 (Wb/Bm) | 21,8 [1–78]/60µL | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | 2.6–80% (after 4–7 wks) |
| | 11 (Wb/Bm) | 31.0 [1–106]/60µL | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | **4.7–20.8%** (after 3–9 wks) |
| | 9 (Wb/Bm) | 20.5 [2–79]/60µL | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | 2.9–9.1% (after 3–24 wks) |
| | 6 (Wb/Bm) | 5.2 [2–10]/60µL | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | 0 (after 1–8 wks) |
| | 10 (Wb/Bm) | 32.4 [5–152]/60µL | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | 0.7 –20% (after 1–6 wks) |

**Figure 23. Summary on the efficacy of levamisole alone on lymphatic filariae (*Wuchereria bancrofti* or *Brugia malayi*)**

## 5.4.3 Methods

### *5.4.3.1 Study design*

This adaptive double-blind, randomized, placebo-controlled trial included 3 independent cohorts with ascending *Loa* MFD. Recruitment to the next cohort started if no SAE occurred in the previous cohort.

To assess the safety of LEV, cohort 1 was composed of participants with low MFD (1-1999 mfs/mL) in whom low doses of LEV were tested. Participants were allocated to one of 3 arms: LEV 1 mg/kg (LEV-1.0), LEV 1.5 mg/kg (LEV-1.5) or placebo. After confirmation that these doses of LEV were well tolerated in patients with low MFD, an independent Data Safety Monitoring Board (DSMB) reviewed the safety and efficacy results to determine whether the dose could be increased for the next cohorts. Following the DSMB recommendations, two cohorts, each comprising two parallel arms (single dose of LEV at 2.5 mg/kg [LEV-2.5], or matched placebo) were launched: cohort 2 included subjects with MFD between 1 and 14 999 mfs/mL, and cohort 3 included all microfilaremic subjects without upper limit of MFD.

To assess efficacy, *Loa* MFD was measured 5 days before treatment (D-5), and at day 2 (D2), day 7 (D7) and day 30 (D30) post-treatment. At D-5, all participants underwent a medical examination and a questionnaire to check for inclusion and exclusion criteria (see below). At D2 and D7, each participant underwent a medical examination and screening for any adverse events (AEs). A medical team visited the villages of all participants every day from D0 to D7 to manage AEs. All subjects received a participant card with emergency contact information.

### *5.4.3.2 Study area and selection of participants*

Participants were recruited in 21 villages located within 40 kilometers of Sibiti (3°41’00’’S, 13°21’48’’E), the capital town of the Congolese administrative department of Lékoumou, a forested area where loiasis is endemic.

Participants were identified in two steps. In November 2019, residents were invited to participate in a survey to screen the population for loiasis. Due to the Covid-19 pandemic, the launch of the trial testing had to be postponed to mid-January 2021. At that date, those subjects

who were found microfilaremic in 2019, were aged 18-65, and weighed 50-85 kg for women or 45-85 kg for men, were invited to be reexamined to assess their eligibility to participate in the trial.

Volunteers underwent a medical evaluation and those with past or current history of neurological or neuropsychiatric disorders, or physical symptoms suggesting systemic disorders, were excluded from recruitment. People treated with clozapine, phenothiazines, sulfasalazine, carbamazepine, antithyroid drugs, ticlopidine, cimetidine, warfarin or gold salts were also excluded due to a possible drug interaction with LEV. Women who reported being pregnant for less than three months, people with acute infection requiring treatment within the 10 days preceding the trial, and people who had received IVM, albendazole or LEV during the previous six months were also excluded.

The clinical trial was conducted from January to April 2021.

#### *5.4.3.3 Randomization, blinding and drug preparation*

For the first cohort, a 1:1:1 randomization of 3 arms with blocks size of 6 was performed. For the second and third cohorts, a 1:1 randomization of 2 arms with blocks size of 4 was used. All randomizations were done by an independent statistician and stratified by gender and median age.

Sealed envelopes were prepared either with 5 placebo tablets or matched tablets of LEV 10 mg, LEV 50 mg or placebo according to participants' weights. Tablets were swallowed under the supervision of a single physician. All tablets were purchased from ACE Pharmaceuticals BV (Zeewolde, The Netherlands).

#### *5.4.3.4 Laboratory procedures*

The *Loa* MFD was assessed by examining two 50 µL calibrated blood smears (CBS1 and CBS2) at D-5, D2, D7 and D30. All CBSs were prepared with blood taken between 10:00 AM and 3:00 PM to account for the diurnal periodicity of *Loa* mfs in peripheral blood (Kamgno et al. 2009). In addition, CBSs for a given participant were prepared at the same time of the day on D-5, D2, D7 and D30. As it is known that temperature can influence *Loa* MFD (Hawking et al. 1967), the ambient and subjects' body temperatures at the time of sampling were recorded using electronic thermometers. Blood was collected by finger-prick and spread on two labelled slides. The slides

were dried at ambient temperature, dehemoglobinized and stained with Giemsa within 4 hours. Each slide was read independently by two experienced biologists who were blinded to treatment. All *Loa* mfs were counted using a microscope at 100X magnification. Slides with a MFD difference exceeding 10% between the two readings were re-read blind to the first result. The arithmetic means of the MFDs measured at the four readings (CBS1 by readers 1 and 2, CBS2 by readers 1 and 2) were used for the analyses, the results being expressed in mfs/mL.

#### *5.4.3.5 Objectives and outcome measures*

The primary objective of the trial was to evaluate the safety of single-dose LEV in individuals with *Loa* microfilaremia. The primary outcome measures were (i) the occurrence of an SAE and (ii) the frequency of AEs during the first week post-treatment. AEs are classified according to the following method:

Any reaction occurring in a person who is a subject of research involving the human subject whether or not the event is related to the research or to the administration of the drug is defined as an adverse event (AE). Any harmful and undesired reaction following administration of the drug, or any incident that could have resulted in such a reaction if appropriate action had not been taken, in an individual who is a research subject is defined as an adverse drug reaction (ADR). Any AE or ADR meets the definition of "serious" if it results in death, endangers the life of the participant, requires hospitalization or prolonged hospitalization; causes significant or lasting disability or incapacity; results in a congenital anomaly or malformation or is considered by investigators as a significant medical event. The intensity of all AEs (serious and non-serious) has been assessed according to the ICH guidelines: mild, moderate, severe or life-threatening (ICH 2016).

The intensity of all clinical AEs (severe and non-serious) has been assessed according to the following list:

- Grade 1 Mild or transient discomfort, without limitation of usual daily activity; does not require medical intervention or corrective treatment.
- Grade 2 Moderate Partial limitation of usual daily activity; medical intervention or corrective medical intervention or corrective treatment may not be necessary.
- Grade 3 Severe Limitation of usual daily activity; requires medical intervention and corrective treatment, hospital admission possible.
- Grade 4 Life-threatening Very limited activity; requires medical intervention and corrective treatment, almost always in hospital.

However, biological AEs found on further investigation in the event of an SAE will be assessed according to the CTCAE grading scale version 5.0 and reported by the investigator in the case report form.

The secondary objective was to assess the effect of LEV on *Loa* MFD measured by: (i) the MFD reduction rates at D2, D7 and D30 and (ii) the proportions of subjects with a MFD reduction rate ≥40% and/or ≥ 80% at D2, D7 and D30. Reduction rates were calculated as follows: ((MFD at D-5) - (MFD at DX))/(MFD at D-5) with X=2, 7 or 30.

#### *5.4.3.6 Sample size calculation*

As no case of SAE has ever been reported after LEV treatment in Central Africa, sample size calculations were performed using theoretical efficacy levels based on results of its effect on other filariasis. We made the hypothesis that <10% participants treated with placebo but ≥40% participants treated by LEV would have an MFD reduction rate exceeding 40% at D7. A sample size of 36 individuals per arm warrants an 80% power to detect a between-treatment difference at a 5% significance level. Assuming that 10% of enrolled subjects would be lost to follow-up at D7, a minimum of 40 participants had to be included in each arm.

#### *5.4.3.7 Statistical analysis*

For the safety analyses, the numbers and proportions of participants with AEs were tabulated by AE severity score and arms. For the efficacy analyses, the arithmetic means and medians of individual MFD reduction rates were calculated and compared between arms at D2, D7 and D30, using Kruskal-Wallis test (KW test) and ANOVA. The proportions of participants with MFD reduction exceeding 40% and 80% were compared between arms with Fisher's exact tests at D2, D7 and D30.

Cohorts 2 and 3 were pooled in order to increase statistical power because no significant baseline differences between arms were found in each of these cohorts.

As secondary analyses, we performed a linear regression using the absolute difference in MFD between D-5 and D2 to assess the effects of the subjects' body temperature, the ambient temperatures, and sampling time differences between D-5 and D2. A random effect was set on areas of residence.

Finally, taking the MFD reduction rate (<40% vs. ≥40%) as dependent variable, we performed logistic regressions to evaluate whether the subjects' body temperature, the ambient temperature at sampling time, and the difference between the time of samplings at D-5 and DX had an effect on the MFD results. A random effect was set on areas of residence.

All statistical analyses were performed using Stata 15 (StatCorps LP, College Station, Texas, USA).

#### *5.4.3.8 Trial registration and ethic statement*

This study was approved by the Committee on Ethics in Health Sciences Research (N° 226/MRSIT/IRSSA/CERRSSA) and an Administrative Authorization (N° 469/MSP/CAB/UCPP-19) was released by the Ministry of Health and Population of the Republic of the Congo. This study was conducted in accordance with the rules of Good Clinical Practices.

All participants signed an informed consent form before initiation of any study-related procedure. This trial is registered as number NCT04049630 in https://clinicaltrials.gov/.

### 5.4.4 Results

#### *5.4.4.1 Screening of eligible participants*

A total of 2052 individuals screened in 2019 met the age and weight eligibility criteria and 389 of them (18.9%, 264 males and 125 females) had *Loa* mfs in their blood. Among these 389 subjects, 344 were still microfilaremic in 2021 (88.4%).

#### *5.4.4.2 Baseline characteristics*

After checking for inclusion and exclusion criteria, 81 participants were randomly assigned to cohort 1 (1-1999 mfs/mL), 111 to cohort 2 (1-14 999 mfs/mL) and 63 to cohort 3 (positive MFD with no upper limit) (Figure 24). Considering all cohorts together, 112 subjects had a *Loa* MFD of 1-1999 mfs/mL, 106 an MFD of 200-14 999 mfs/mL and 33 an MFD ≥15 000 mfs/mL.

The baseline characteristics of participants are shown in Table 36. Within each cohort, there was no difference between arms regarding age distribution, sex ratio, nor mean and median *Loa* MFD.

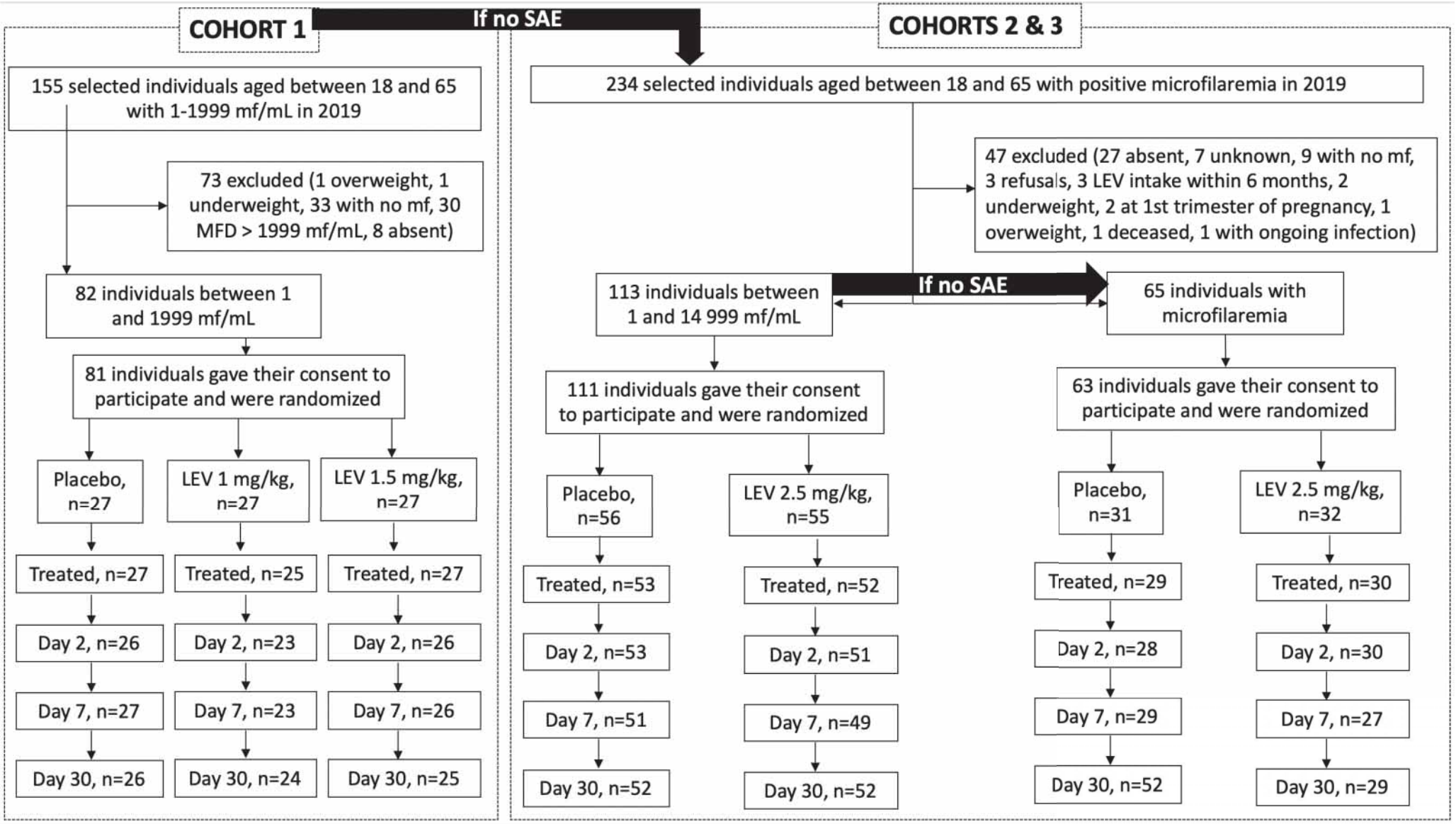


**Figure 24. Flowchart of the clinical trial**

| | Cohort 1 | | | Cohort 2 | | Cohort 3 | | Cohorts 2 & 3 | |
|---|---|---|---|---|---|---|---|---|---|
| | **Placebo** | **Levamisole 1 mg/kg** | **Levamisole 1.5 mg/kg** | **Placebo** | **Levamisole 2.5 mg/kg** | **Placebo** | **Levamisole 2.5 mg/kg** | **Placebo** | **Levamisole 2.5 mg/kg** |
| **Sex** | | | | | | | | | |
| Female | 9 | 10 | 10 | 15 | 16 | 7 | 7 | 22 | 23 |
| Male | 18 | 17 | 17 | 39 | 37 | 24 | 25 | 63 | 62 |
| **Age (**mean ± sd) | 47.6 ± 15.0 | 47.5 ± 14.9 | 47.7 ± 13.6 | 47.3 ± 11.8 | 48.0 ± 12.9 | 45.9 ± 13.2 | 46.5 ± 12.4 | 46.8 ± 12.3 | 47.4 ±12.7 |
| **Microfilaremia** (mf/ml) | | | | | | | | | |
| Arithmetic mean ± sd | 636 ± 575 | 641 ± 536 | 641 ± 549 | 5082 ± 3826 | 5104 ± 3843 | 23 356 ± 19 714 | 18 146 ± 15 843 | 11 468 ± 14 799 | 9817 ± 11 742 |
| Minimum; Maximum | 15; 1995 | 15; 1995 | 60; 1975 | 10; 14 015 | 5; 13 850 | 255; 69 085 | 940; 60 920 | 5; 69 085 | 10; 60 920 |
| Geometric mean [95% CI] | 350 [204–600] | 412 [265–642] | 359 [210–614] | 2916 [1938–4388] | 2832 [1816–4416] | 13 320 [7936–22 355] | 10 927 [7074–16 879] | 4957 [3483–7055] | 4614 [3255–6540] |
| Median [ITQ] | 470 [175–885] | 510 [180–920] | 475 [145–915] | 4362 [2090–7500] | 4075 [2035–7150] | 16 370 [9035–34 120] | 11 450 [6160–30 000] | 6070 [2415–13 185] | 6160 [2750–112 45] |
| ***Mansonella perstans*** Prevalence (N; %) | 2; 7.4% | 3; 11.1% | 3; 11.1% | 7; 13.0% | 9; 17.0% | 5; 16.1% | 4; 12.5% | 12; 14.4% | 13; 15.3% |
| **Heart rate** (mean [bpm] ± sd) | 72.2 ± 12.6 | 75.8 ± 13.0 | 69.8 ± 10.2 | 78.6 ±12.3 | 76.5 ± 13.7 | 72.3 ± 13.0 | 77.0 ± 10.2 | 76.4 ± 12.8 | 76.6 ± 12.5 |
| **Mean blood pressure** (mean [mmHg] ± sd) | 101 ± 18 | 104 ± 15 | 99 ± 13 | 105 ± 17 | 105 ± 14 | 105 ± 18 | 107 ± 16 | 105 ± 17 | 105 ± 15 |
| **Systolic blood pressure** (mean [mmHg] ± sd) | 128 ± 21 | 131 ± 20 | 127 ± 21 | 132 ± 22 | 130 ± 20 | 132 ± 23 | 133 ± 21 | 132 ± 23 | 131 ± 20 |
| **Diastolic blood pressure** (mean [mmHg] ± sd) | 74 ± 12 | 76 ± 12 | 71 ± 9 | 78 ± 14 | 79 ± 11 | 77 ± 17 | 81 ± 13 | 78 ± 15 | 80 ± 11 |
| **Body temperature** (mean [°C] ± sd) | 36.2 ± 0.7 | 36.4 ± 0.6 | 36.4 ± 0.6 | 36.6 ± 0.3 | 36.7 ± 0.3 | 36.5 ± 0.2 | 36.5 ± 0.3 | 36.6 ± 0.3 | 36.6 ± 0.3 |

**Tableau 36. Baseline (pre-treatment) characteristics of trial participants**

sd, standard deviation; bpm, beats per minute; 95% CI, 95% confidence intervals. ITQ, interquartile range

#### *5.4.4.3 Results of low doses of LEV in subjects with low Loa MFD*

The first cohort included participants with MFD < 2000 mfs/mL. No SAE related to treatment occurred. Thirteen patients reported 15 AEs (4 in the LEV-1.0 arm, 4 in the LEV- 1.5 arm and 5 in the placebo arm). Among the AEs reported in the LEV-1.0 arm, 2 were mild (1 epigastralgia and 1 edema) and 2 were moderate (2 cases of generalized pruritus) and required symptomatic treatment (antihistamines and corticosteroids). Among the AEs reported in the LEV-1.5 arm, 2 were not related to treatment (1 murder and 1 malaria attack) and 2 were mild (2 cases of localized pruritus). Among the AEs reported in the placebo arm, 1 was not related to the treatment (malaria attack), 2 were mild (2 cases of dizziness) and 2 were moderate (1 generalized pruritus and 1 epigastralgia) and required symptomatic treatment (antihistamines and proton pump inhibitor, respectively). The proportions of AEs did not differ between the three arms (Fisher exact test, *P* = 1.000).

Neither the mean and median MFDs (Table 37) nor the proportion of participants with a 40% or 80% MFD reduction (Table 38) were significantly different between the three arms at D2, D7 and D30.

| | LEV 1.5 mg/kg | | | LEV 1 mg/kg | | | Placebo | | | *P* * | *P* ** |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | MFD arithmetic mean | Mean relative difference | Median relative difference | MFD arithmetic mean | Mean relative difference | Median relative difference | MFD arithmetic mean | Mean relative difference | Median relative difference | | |
| **Day 2** | 587.5 mf/mL | +0.7% ± 40.7% | –13.4% [–27.8%; +33.9%] | 792.4 mf/mL | +17.1% ± 72.7 % | +3.1% [–35.4%; +40.9%] | 600.3 mf/mL | +8.2% ± 96.6% | –2.5% [–35.9%; +34.3%] | .952 | .738 |
| **Day 7** | 679.8 mf/mL | +33.5% ± 116.5% | +13.4% [–35.2%; +52.7%] | 869.8 mf/mL | +27.9% ± 60.0% | +19.7% [–20.9%; +61.9%] | 524.4 mf/mL | +4.6% ± 117.0% | –20.0% [–53.8%; +22.3%] | .036 | .559 |
| **Day 30** | 648.6 mf/mL | +15.8% ± 58.4% | +3.8% [–18.6%; +20.0%] | 704.4 mf/mL | +23.0% ± 80.3% | +2.2% [–35.8%; +75.5%] | 536.0 mf/mL | –5.3% ± 93.7% | –23.3% [–49.5%; +13.8%] | .107 | .563 |

**Tableau 37. Mean microfilaremia, mean and median relative difference in microfilaremia between D-5 and DX (X=2, 7 or 30), by arm (cohort 1)**

* Kruskal-Wallis test; ** ANOVA

| | 40% decrease in microfilaremia | | | | | | | 80% decrease in microfilaremia | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | LEV 1.5 mg/kg | | LEV 1 mg/kg | | Placebo | | *P* * | LEV 1.5 mg/kg | | LEV 1 mg/kg | | Placebo | | *P* * |
| | Yes | No | Yes | No | Yes | No | | Yes | No | Yes | No | Yes | No | |
| **Day 2 (N, %)** | 4 (15.4%) | 22 (84.6%) | 4 (17.4%) | 19 (82.6%) | 5 (19.2%) | 21 (80.8%) | 1.000 | 0 (0%) | 26 (100%) | 0 | 23 (100%) | 3 (11.5%) | 23 (88.5%) | .103 |
| **Day 7 (N, %)** | 5 (19.2%) | 21 (80.8%) | 2 (8.7%) | 21 (91.3%) | 9 (33.3%) | 18 (66.7%) | 0.108 | 0 (0%) | 26 (100%) | 1 (4.4%) | 25 (95.6%) | 3 (11.1%) | 24 (88.9%) | .204 |
| **Day 30 (N, %)** | 4 (15.4%) | 22 (84.6%) | 8 (33.3%) | 16 (66.7%) | 12 (48.0%) | 13 (52.0%) | 0.047 | 0 (0%) | 26 (100%) | 1 (4.2%) | 23 (95.8%) | 4 (16.0%) | 21 (84.0%) | .058 |

**Tableau 38. Proportion of participants with a 40% and 80% reduction in their microfilaremia per treatment arm in cohort 1**

* Fisher exact test

### *5.4.4.4 Safety of treatment with a single dose of LEV at 2.5 mg/kg*

In cohorts 2 and 3, no SAE occurred. A total of 17 AEs were reported. Among them, 4 were not related to the trial (2 malaria attacks, 1 post-traumatic edema and 1 scalp furuncle). Of the 13 AEs possibly associated with the experimental drug, 3 occurred in the placebo arm and 10 in the LEV arm (Fisher's exact test, *P* = .018). All AEs reported were mild and transient. Table 39 summarizes the AEs possibly related to LEV in the two cohorts. In the LEV-2.5 arm, the mean initial MFDs were 19 645 [4920-60 920] mfs/mL and 9877 [10-49 605] mfs/mL in participants who reported an AE and in those who did not, respectively. This difference was significant (KW test, *P* = .020).

| Treatment | Adverse event | Gradation | Baseline MFD (mf/mL) | Days post-treatment | Absolute and relative difference in MFD from baseline to Day 2 |
|---|---|---|---|---|---|
| Placebo | Dizziness | Mild | 2305 | 1 | – 1595 mf/mL (– 69.2%) |
| Placebo | Pruritus | Mild | 5990 | 2 | – 210 mf/mL (– 3.5%) |
| Placebo | Conjunctivitis | Mild | 36 990 | 0 | + 1738 mf/mL (+ 4.7%) |
| LEV 2.5 mg/kg | Conjunctivitis | Mild | 4920 | 2 | – 763 mf/mL (– 15.5%) |
| LEV 2.5 mg/kg | Blepharitis | Mild | 4920 | 3 | – 763 mf/mL (– 15.5%) |
| LEV 2.5 mg/kg | Pruritus | Mild | 5985 | 0 | – 180 mf/mL (– 3.0%) |
| LEV 2.5 mg/kg | Dizziness | Mild | 6160 | 0 | – 1337 mf/mL (– 21.7%) |
| LEV 2.5 mg/kg | Vomiting | Mild | 7330 | 0 | – 3665 mf/mL (– 50.0%) |
| LEV 2.5 mg/kg | Dizziness | Mild | 24 420 | 0 | + 11 843 mf/mL (+ 48.5%) |
| LEV 2.5 mg/kg | Dizziness | Mild | 30 000 | 0 | – 4170 mf/mL (– 13.9%) |
| LEV 2.5 mg/kg | Dizziness | Mild | 32 090 | 0 | – 8664 mf/mL (+ 27.0%) |
| LEV 2.5 mg/kg | Epigastralgia | Mild | 30 950 | 0 | – 9130 mf/mL (– 29.5%) |
| LEV 2.5 mg/kg | Vomiting | Mild | 60 920 | 0 | – 15 473 mf/mL (– 25.4%) |

**Tableau 39. Adverse events possibly related to treatment in cohorts 2 and 3**

### *5.4.4.5 Effect of LEV 2.5 mg/kg on Loa MFD*

Median MFD were significantly lower in the LEV group than in the placebo arm at D2, D7 and D30 (KW test, *P* = .001, .001 and .036, respectively). This effect was particularly clear in individuals with high baseline MFD (Table 40). As shown in Figure 25, the arithmetic mean, the geometric mean, and the median of the MFD increase over time in the placebo group while they decrease at D2 and then increase again at D7 and D30 in the LEV arm. The high interindividual variability in response to treatment, which can be seen in Figure 26, is confirmed by the high standard errors in Table 40, notably among those with low initial MFD. The proportion of participants with an 80% reduction in MFD did not differ significantly between treatment arms at D2, D7 and D30. The proportion of participants with a 40% reduction was significantly higher in the LEV arm compared to the placebo arm at D2 (*P* < .001) but not at D7 (*P* = .269) or D30 (*P* = .107) (Table 41).

The proportions of participants with less than 30 minutes difference between the time of their pre-treatment CBS and those prepared at D2, D7 and D30 were 93.6%, 94.4% and 98.1%, respectively.

Analyses adjusting for body and ambient temperatures, baseline MFD and village of residence showed that people treated with LEV-2.5 had a mean MFD reduction of 2222 mf/mL at D2, compared to the placebo group (*P* < .001) (Table 42). The relative difference in MFD between baseline and D2 is dependent of the baseline MFD. Adjusted analyses also showed that people treated with LEV-2.5 were 18.9 times more likely to have a 40% decrease in their microfilaremia at D2 than the placebo-treated subjects (*P* = .005). No between-group difference was found at D7 (adjusted Odds-ratio [aOR] = 1.9; *P* = .320) or D30 (aOR = 2.7; *P* = .051) (Table 43). Fractional polynomial regression of degree 4 (Akaike Information Criterion [AIC] = 784) seems to be the best approach to model this phenomenon (AIC = 784). Linear (AIC = 790), quadratic (AIC = 791) and piecewise polynomial regression (AIC = 794) regressions may also be applicable (Figure 27).

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| | | LEV 2.5 mg/kg | | | Placebo | | | *P* * | *P* ** |
|---|---|---|---|---|---|---|---|---|---|
| | | **Mean MFD** | **Mean relative difference** | **Median relative difference** | **Mean MFD** | **Mean relative difference** | **Median relative difference** | | |
| **Day 2** | **All participants** | **8509 mf/mL** | **+6.1% ± 108.6%** | **–12.9% [–32.4%; +19.7%]** | **12 443 mf/mL** | **+33.0% ± 82.3%** | **+15.5% [–9.4%; +43.4%]** | **.078** | **.001** |
| | 1-2499 mf/mL | | +40.7% ± 212.8% | –6.1% [–34.6%; +26.0%] | | +76.4% ± 147.3% | +17.8% [–4.5%; +88.9%] | .545 | .087 |
| | 2500-6999 mf/mL | | +6.7% ± 46.8% | –4.8% [–27.4%; +46.7%] | | +33.2% ± 47.0% | +23.4% [–0.4%; +63.3%] | .075 | .047 |
| | 7000-11 999 mf/mL | | –3.7% ± 30.5% | –3.6% [–23.0%; +17.7%] | | +23.0% ± 25.3% | +19.3% [+3.6%; +39.4%] | .005 | .007 |
| | ≥12 000 mf/mL | | –19.5% ± 30.3% | –25.2% [–40.5%; –13.9%] | | +2.02% ± 27.1% | –8.2% [–19.0%; +19.0%] | .023 | .008 |
| **Day 7** | **All participants** | **8847 mf/mL** | **+13.9% ± 122.3%** | **–4.9% [–31.1%; +22.5%]** | **12 879 mf/mL** | **+29.2% ± 76.6%** | **+18.7% [–2.0%; +47.8%]** | **.347** | **.001** |
| | 1-2499 mf/mL | | +47.1% ± 234.3% | +4.3% [–31.1%; +27.8%] | | +62.9% ± 136.6% | +34.1% [–7.0%; +78.4%] | .797 | .084 |
| | 2500-6999 mf/mL | | +12.3% ± 56.8% | –0.5% [–24.6%; +63.3%] | | +36.8% ± 45.8% | +30.8% [+7.6%; +62.4%] | .141 | .053 |
| | 7000-11 999 mf/mL | | +7.3% ± 36.0% | +4.1% [–19.7%; +38.7%] | | +13.8% ± 29.0% | +19.9% [+1.5%; +26.6%] | .548 | .412 |
| | ≥12 000 mf/mL | | –14.1% ± 23.1% | –10.9% [–36.0%; –4.6%] | | +5.5% ± 26.9% | +3.1% [–13.7%; +19.8%] | .022 | .020 |
| **Day 30** | **All participants** | **10 241 mf/mL** | **+55.6% ± 395.2 %** | **–0.5% [–26.6%; +24.6%]** | **13 364 mf/mL** | **+29.9% ± 86.6%** | **+13.5% [–7.2%; +35.2%]** | **.568** | **.036** |
| | 1-2499 mf/mL | | +231.0% ± 805.1% | +9.1% [–33.9%; +81.5%] | | +67.7% ± 157.1% | +9.0% [–26.6%; +120.7%] | .367 | .989 |
| | 2500-6999 mf/mL | | –4.0% ± 35.5% | –7.2% [–29.5%; +15.9%] | | +20.8% ± 43.4% | +17.4% [–9.0%; +31.3%] | .048 | .030 |
| | 7000-11 999 mf/mL | | +11.4% ± 25.7% | +10.1% [–5.9%; +24.6%] | | +15.2% ± 29.8% | +13.5% [+1.8%; +34.5%] | .674 | .673 |
| | ≥12 000 mf/mL | | –1.8% ± 31.0% | –9.0% [–26.9%; +16.0%] | | +14.2% ± 26.4% | +16.4% [–6.5%; +25.1%] | .081 | .036 |

**Tableau 40. Mean microfilaremia, mean and median relative difference in microfilaremia by between D-5 and DX (X=2, 7 or 30), by arm (cohort 2 and 3) and initial MFD stratum**

Initial MFD is stratified according to interquartile range

* ANOVA; ** Kruskal-Wallis test

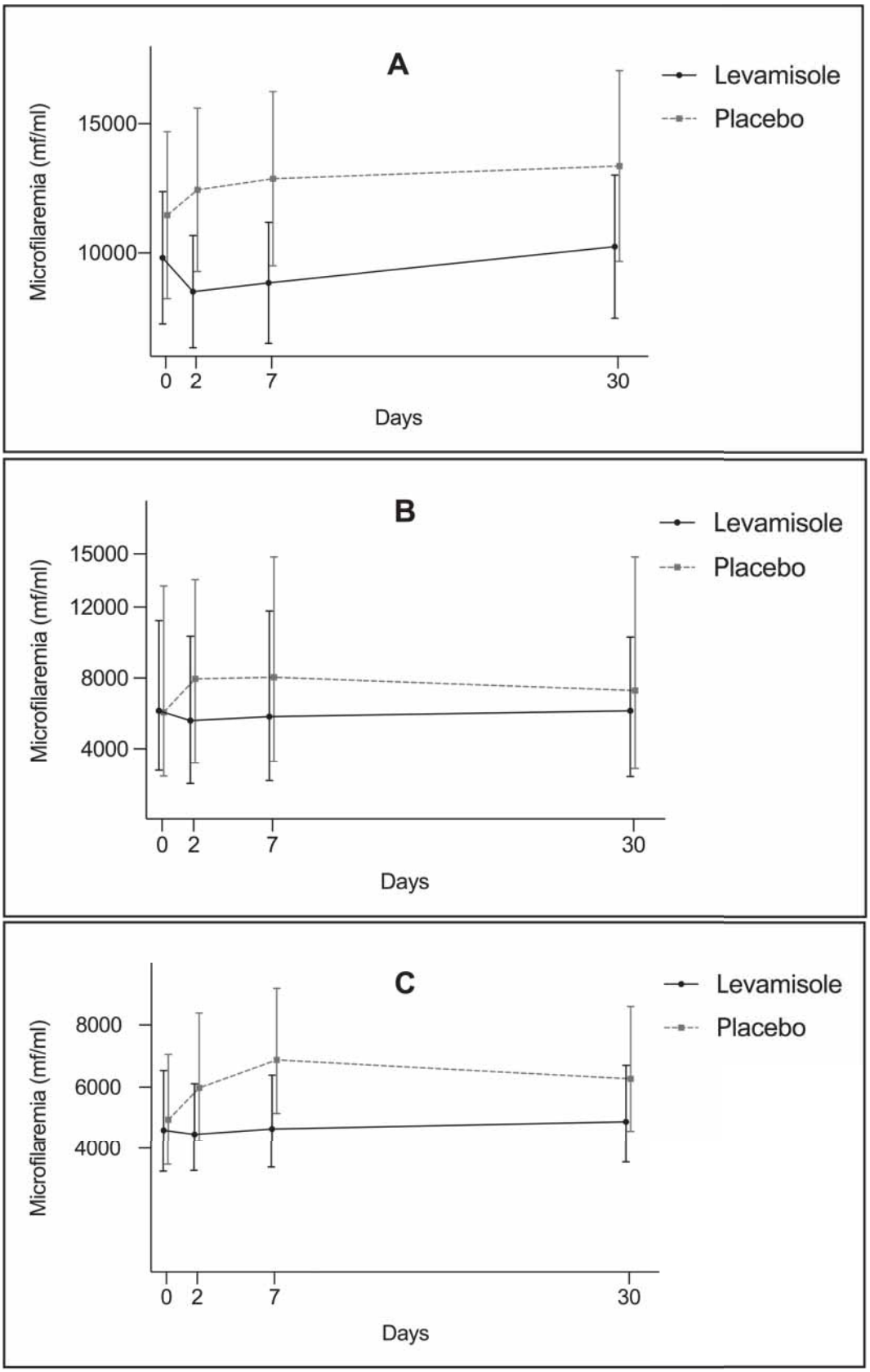


**Figure 25. Evolution of mean and median microfilarial densities**

A, arithmetic mean evolution with 95% confidence intervals;

B, median evolution with interquartile range;

C, geometric mean evolution with 95% confidence intervals

| | 40% decrease in microfilaremia | | | | | 80% decrease in microfilaremia | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | LEV 2.5 mg/kg | | Placebo | | *P* * | LEV 2.5 mg/kg | | Placebo | | *P* * |
| | Yes | No | Yes | No | | Yes | No | Yes | No | |
| **Day 2 (N, %)** | 14 (17.5%) | 66 (82.5%) | 1 (1.2%) | 80 (98.8%) | < .001 | 1 (1.3%) | 79 (98.7%) | 0 (0%) | 81 (100%) | .497 |
| **Day 7 (N, %)** | 9 (11.8%) | 67 (88.2%) | 5 (6.3%) | 75 (93.7%) | .269 | 1 (1.3%) | 75 (98.7%) | 1 (1.3%) | 79 (98.7%) | 1.000 |
| **Day 30 (N, %)** | 15 (18.5%) | 66 (81.5%) | 7 (8.6%) | 74 (91.4%) | .107 | 0 (0%) | 81 (100%) | 1 (1.3%) | 80 (98.7%) | 1.000 |

**Tableau 41. Proportion of participants with a 40% and 80% reduction in their microfilaremia per treatment arm in cohorts 2 & 3**

* Fisher exact test

| | cCoef | 95% CI | *P* | aCoef | 95% CI | P |
|---|---|---|---|---|---|---|
| **Treatment** | | | | | | |
| Placebo | Ref. | | | Ref. | | |
| LEV 2.5 mg/kg | - 2058 | -3425 – -690 | 0.003 | - 2222 | -3484 – -959 | 0.001 |
| **Baseline MFD** | | | | | | |
| 1–2499 mf/ml | Ref. | | | Ref. | | |
| 2500–6499 mf/ml | + 692 | -1170 – +2554 | 0.466 | + 523 | -1253 – +2300 | 0.564 |
| 6500–11.999 mf/ml | + 564 | -1320 – +2448 | 0.557 | + 531 | -1280 – +2342 | 0.565 |
| > 12.000 mf/ml | - 3107 | -4992 – -1223 | 0.001 | - 3051 | -4860 – -1242 | 0.001 |
| **Temperature difference** | | | | | | |
| Btw -2°C & +2°C | Ref. | | | Ref. | | |
| +2°C or more | + 86 | -2822 – +2994 | 0.954 | - 153 | -2960 – +2653 | 0.915 |
| **Body temperature difference** (continuous; °C) | +1314 | -358 – 2986 | 0.124 | + 883 | -690 – +2458 | 0.271 |
| **Random effect on area of residence** | | | ICC: 29.6% | | | 0.065 |

**Tableau 42. Mixed linear regression for the absolute difference between baseline and Day 2**

cCoef, crude coefficient; aCoef, adjusted coefficient 95% CI, 95% confidence intervals; Btw, between; ICC: intraclass correlation (calculated on full model)
* No 40%-decrease in MFD in "> 15 minutes group".
** No 40%-decrease in MFD in "-2°C or less group"
*** 40%-decrease in MFD only in "between-2°C and +2°C group"

| | Day 2 | | | | | | Day 7 | | | | | | Day 30 | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | **cOR** | **95% CI** | ***P*** | **aOR** | **95% CI** | **P** | **cOR** | **95% CI** | ***P*** | **aOR** | **95% CI** | **P** | **cOR** | **95% CI** | ***P*** | **aOR** | **95% CI** | **P** |
| **Treatment** | | | | | | | | | | | | | | | | | | |
| Placebo | Ref. | | | Ref. | | | Ref. | | | Ref. | | | Ref. | | | Ref. | | |
| LEV 2.5 mg/kg | 16.7 | 2.2 – 132 | 0.007 | 26.6 | 2.9 – 237 | 0.003 | 2.0 | 0.6 – 6.3 | 0.229 | 1.9 | 0.5 – 7.2 | 0.320 | 2.4 | 0.9 – 6.2 | 0.072 | 2.7 | 1.0 – 7.1 | 0.051 |
| **Baseline MFD (mf/mL)** | | | | | | | | | | | | | | | | | | |
| 1–2499 | 1.0 | 0.3 – 3.9 | 0.966 | 0.8 | 0.1 – 4.4 | 0.761 | 5.6 | 0.6 – 50 | 0.125 | 8.0 | 0.8 – 80 | 0.078 | 2.4 | 0.7 – 7.8 | 0.145 | 2.5 | 0.7 – 8.2 | 0.140 |
| 2500–6499 | 0.4 | 0.1 – 1.9 | 0.227 | 0.2 | 0.1 – 1.5 | 0.153 | 5.3 | 0.6 – 47 | 0.137 | 5.1 | 0.5 – 51 | 0.170 | 1.0 | 0.2 – 3.6 | 0.968 | 0.9 | 0.2 – 3.5 | 0.895 |
| 6500–11 999 | 0.6 | 0.1 – 2.5 | 0.460 | 0.4 | 0.1 – 2.4 | 0.311 | 3.3 | 0.3 – 33 | 0.305 | 1.3 | 0.1 – 22 | 0.865 | 0.4 | 0.1 – 2.1 | 0.277 | 0.4 | 0.1 – 2.0 | 0.242 |
| > 12.000 | Ref. | | | Ref. | | | Ref. | | | Ref. | | | Ref. | | | Ref. | | |
| **Time difference** | | | | | | | | | | | | | | | | | | |
| ≤ 15 minutes | . | | | . | | | Ref. | | | Ref. | | | Ref. | | | Ref. | | |
| > 15 minutes | Empty* | | | | | | 2.0 | 0.5 – 7.9 | 0.321 | 1.8 | 0.3 – 10.2 | 0.521 | 1.1 | 0.1 – 9.3 | 0.956 | 0.8 | 0.1 – 7.7 | 0.829 |
| **Temperature difference** | | | | | | | | | | | | | | | | | | |
| -2°C or less | Empty** | | | | | | Empty*** | | | | | | No recorded temperatures | | | | | |
| Btw -2°C & +2°C | Ref. | | | Ref. | | | . | | | . | | | | | | | | |
| +2°C or more | 1.1 | 0.1 – 9.2 | 0.939 | 1.9 | 0.1 – 29.5 | 0.656 | Empty*** | | | | | | | | | | | |
| **Body temperature difference** (continuous; °C) | 0.3 | 0.1 – 1.3 | 0.113 | 0.3 | 0.1 – 2.5 | 0.290 | 0.7 | 0.1 –3.5 | 0.671 | 0.3 | 0.1 – 2.2 | 0.248 | | | | | | |
| **Random effect on area of residence** | ICC: 29.6% | | | | | 0.039 | Not significant | | | | | | Not significant | | | | | |

**Tableau 43. Mixed logistic regressions for the 40%-decrease from baseline MFD at Day 2, 7 and 30**

cOR, crude odds-ratio; aOR, adjusted odds-ratio 95% CI, 95% confidence intervals; Btw, between; ICC: intraclass correlation (calculated on full model)

* No 40%-decrease in MFD in "> 15 minutes group".

** No 40%-decrease in MFD in "-2°C or less group"

*** 40%-decrease in MFD only in "between-2°C and +2°C group"

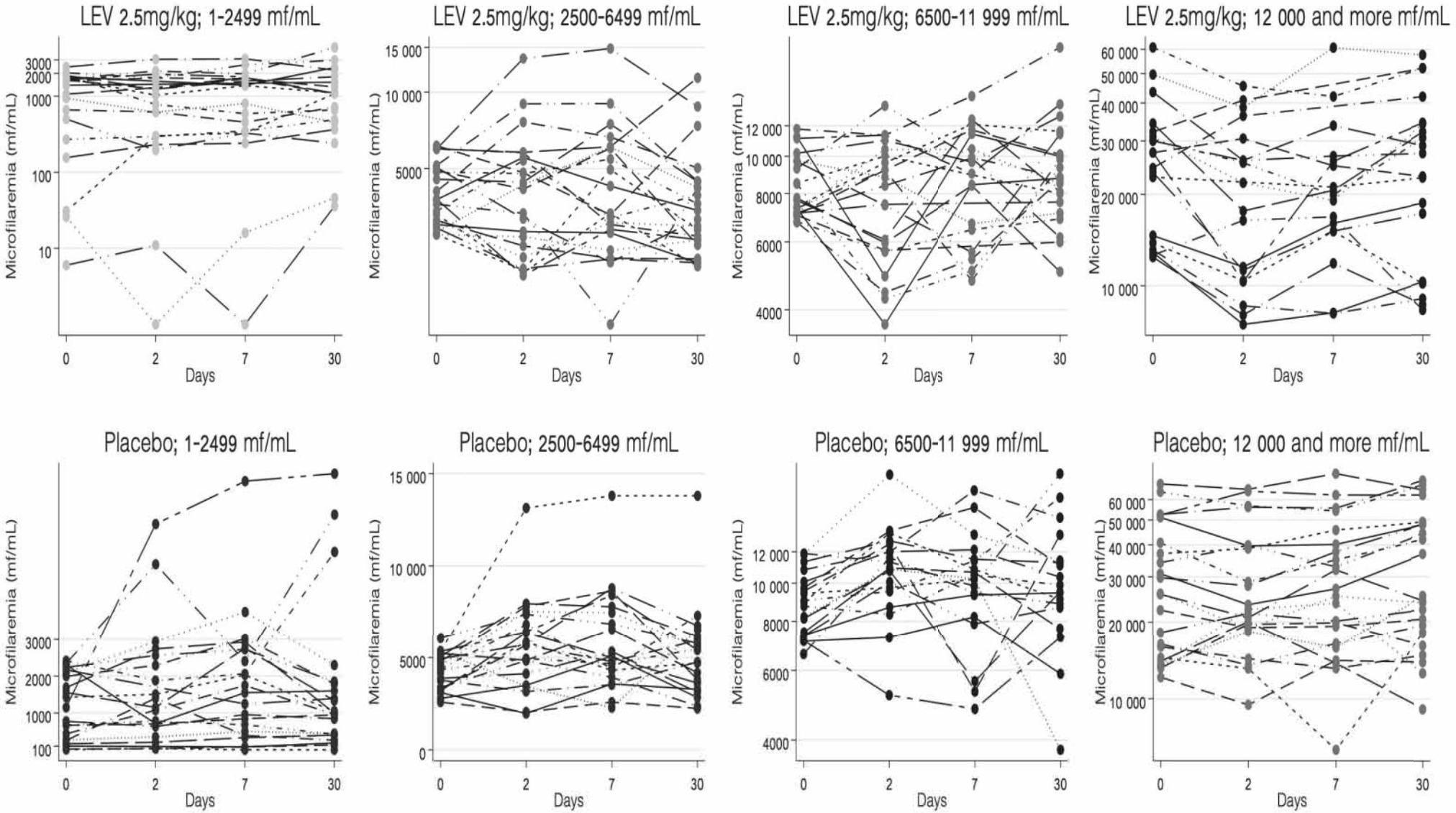


**Figure 26. Evolution of individuals microfilarial densities**

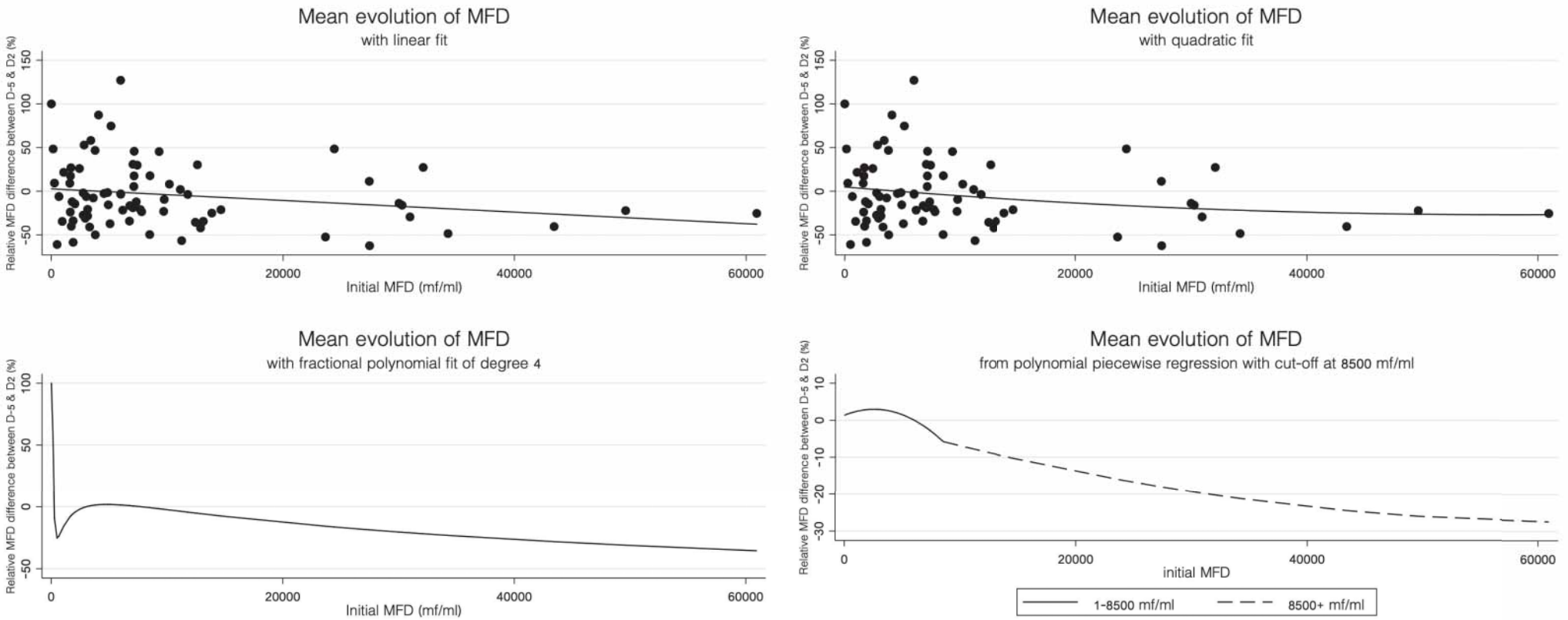


**Figure 27. Predicted relative difference between baseline and Day 2 according to baseline microfilaremia in the Levamisole 2.5 mg/kg group**

With exclusion of outliers due to high variability in low MFD individuals for better fit (when the relative difference is superior at +200% or equals at -100% (only 1 individual with a baseline at 20 mf/ml), N = 78

- **Linear equation (AIC = 790.4):** $MFD\ difference\ between\ D0\ \&\ D2\ (\%) = 2.7 - 6.7 * \frac{Initial\ MFD\ (mf/ml)}{10\,000}$
- **Quadratic equation (AIC = 792.2):** $MFD\ difference\ between\ D0\ \&\ D2\ (\%) = 21.9 - \left(17.3 * \frac{Initial\ MFD\ (mf/ml)}{10\,000}\right) + \left(2.0 * 10^{-4} * \left(\frac{Initial\ MFD\ \left(\frac{mf}{ml}\right)}{10\,000}\right)^2\right)$
- **Fractional polynomial equation of degree 4 (AIC = 785.7):** $MFD\ difference\ between\ D0\ \&\ D2\ (\%) = -2.5 + \left(66.4 * \left(\frac{Initial\ MFD}{10\,000}\right)^{-1.00} - 0.99\right) + \left(7.1 * \left(\frac{Initial\ MFD}{10\,000}\right)^{-1.00} * \ln\left(\left(\frac{Initial\ MFD}{10\,000}\right)\right) - 0.005\right)^2 + \left(65.7 * \left(\left(\frac{Initial\ MFD}{10\,000}\right)^{-0.50} - 0.99\right)^3\right) + \left(81.2 * \left(\left(\frac{Initial\ MFD}{10\,000}\right)^{-0.50} ** ln\left(\left(\frac{Initial\ MFD}{10\,000}\right)\right) - 0.005\right)^4\right)$
- **Piecewise regression (AIC = 794.1):** $MFD\ difference\ between\ D0\ \&\ D2\ (\%) = f(x) = \begin{cases} 1.3 + 12.4 * \left(\frac{Initial\ MFD}{10\,000}\right) - \left(2.4 * 10^{-3} * \left(\frac{Initial\ MFD}{10\,000}\right)^2\right), & x < 8500 \\ 1.3 + \left(12.4 * \left(\frac{Initial\ MFD}{10\,000}\right)\right) - \left(2.4 * 10^{-3} * \left(\frac{Initial\ MFD}{10\,000}\right)\right)^2 + \left(7.0 * 10^{-5} * \left(\frac{Initial\ MFD}{10\,000}\right) * \left(\frac{Initial\ MFD - 8500}{10\,000}\right)\right)^3, & x \geq 8500 \end{cases}$

### 5.4.5 Discussion

This trial is the first to assess the safety and efficacy of LEV in *Loa*-infected subjects. No SAE occurred after a single dose of LEV-1.0, -1.5 or -2.5, even in individuals with high initial MFD. The severity and proportion of AEs observed in the LEV-treated arms were in accordance with those described in the prescription drug information (ACE Phamaceuticals 2018).

The dose recommended to treat soil-transmitted helminthiases (2.5 mg/kg) induced a significant decrease in *Loa* MFD two days post-treatment. Just under 20% of the subjects in the LEV-2.5 arm showed a >40% decrease in their MFD at D2, D7 and D30. At D30, the effect was no longer observed. In the LEV-2.5 arm, only one participant with low baseline MFD (25 mfs/mL) showed an 80% decrease in MFD at D2 and D7; which may be reassuring since a large and rapid effect would raise the question of the occurrence of SAEs similar to those induced after IVM administration in individuals with high *Loa* MFD.

Maximum reduction in *Loa* MFD seems to occur about two days after LEV intake. It is followed by a slight increase in MFD between D2 and D7, and a more marked increase between D7 and D30. This suggests that the mfs are not definitely eliminated and similar results have been reported from trials evaluating LEV on *W. bancrofti* (Moreau et al. 1975). LEV might have a "microfilarifugal" action rather than a microfilaricidal one, i.e. it may stimulate migration of microfilariae to deep organs where they could be sequestered and/or eliminated by the immune system (Mak and Zaman 1980). A second mechanism would be that mfs circulating at the time of treatment are eliminated but are replaced very rapidly by those newly released by adult worms. Mfs might also regain their muscular activity after a phase of temporary paralysis due to several mechanisms (Chandy et al. 2016).

It was observed that individuals who experienced an AE had a higher baseline MFD than those who did not. If AEs result from the elimination or sequestration of large numbers of mfs, then the hypothesis of a rapid reinvasion of the blood stream by new mfs is more likely.

Measurement of MFD varied at the individual level, and was particularly visible in the placebo arm where mean MFD increased over time. This variability has already been described in other trials (Kamgno et al. 2016). This variation may be due to (i) detection error due to variations in the reading of the microscopists in low MFD cases or (ii) heterogeneity in

physiological factors driving MFD variations between subjects. However, we collected two slides for each participant and each slide was read by two different technicians, thus reducing the risk of reading errors. More than 90% of the post-treatment CBSs were prepared within 30 minutes of time of the initial sample, ensuring that the results were not significantly impacted by the diurnal periodicity of *Loa*. Finally, small differences in ambient temperatures on the different sampling days did not significantly impact the results, as shown by the results of the logistic regression.

The results of this trial are promising because they suggest a possible effect of LEV on *Loa* MFD at a dose that is well-tolerated. However, other trials using higher single doses or repeated doses for several days need to be conducted. Should an efficient regimen be identified, the results would enable to determine the optimal time interval between pre-treatment with LEV and safe IVM administration, considering both the pharmacokinetic-pharmacodynamic relationships and logistical constraints (the interval should not exceed a couple of weeks).

### 5.4.6 Conclusion

**Le lévamisole est une molécule qui semble prometteuse, à la fois pour la prise en charge des infections par *L. loa* importées dans les pays du Nord avec de hautes microfilarémies et pour les programmes de lutte contre l'onchocercose. Néanmoins, il s'agit d'une première étude et de nombreuses interrogations restent en suspens : le schéma thérapeutique optimal, le temps optimal entre l'administration de lévamisole et les campagnes d'administration massive de médicaments, par exemple.**

Les essais cliniques portant sur la loase sont compliqués à mettre en place et à en tirer des conclusions pour 3 principales raisons :

- *Loa loa* est un parasite qui possède sa propre périodicité sur une journée, rendant les études longitudinales avec plusieurs points de mesure au cours du suivi compliquées à mettre en place au niveau logistique, puisque les horaires de prélèvements doivent être similaires sur différentes journées afin de pouvoir les comparer.
- Bien que considéré comme relativement stable dans le temps, la microfilarémie de certains individus semble varier dans le temps, à court terme (quelques jours à quelques

mois) et à long terme (plusieurs mois à plusieurs années). Cela implique deux choses : (i) un redépistage systématique de la population microfilarémique pouvant être recensés dans des bases de données avant d'expérimenter une intervention, puisque la microfilarémie aurait pu varier au cours du temps et (ii) une prise en compte de la variabilité « naturelle » de la microfilarémie imposant la présence d'un groupe contrôle sans intervention médicamenteuse via un placebo, par exemple.

- Chaque technique de diagnostic biologique possède une variabilité qui lui est propre. En effet, lors de la lecture des gouttes épaisses calibrées par des microscopistes qualifiées, une variabilité, à la fois intra- et interindividuelle, au niveau des résultats se fait ressentir, contraignant à réaliser de nombreuses lectures supplémentaires. Cette variabilité intra- et interindividuelle n'a cependant jamais été quantifiée de manière conforme aux réglementations COFRAC en vigueur au niveau européen.

Pour mieux comprendre ces phénomènes de périodicité et de variabilité (de microfilarémie et de diagnostic), nous avons réalisé plusieurs études qui seront détaillées au Chapitre 6 :

- La mise en place d'une étude de périodicité sur 13 patients microfilarémiques
- La réutilisation des données de l'essai clinique et du dépistage de 2019 dont sont issus les patients afin de mieux comprendre la variabilité « naturelle » de la loase
- La mise en place d'une étude de laboratoire afin de quantifier la variabilité diagnostique de la goutte épaisse calibrée.

# 6. Périodicité et variabilité de la microfilarémie à *Loa loa*

## 6.1 Contexte et justificatif des études

Les quatre parties qui suivent découlent directement de problématiques auxquelles nous avons été confrontées lors de la mise en place, l'inclusion et le suivi de l'essai clinique du lévamisole sur la loase.

Une des difficultés rencontrées quand on réalise des essais cliniques sur la loase est la périodicité avec laquelle les microfilaires sont présentes dans le sang périphérique. Comme décrit en chapitre 2, cette périodicité engendre plusieurs problématiques :

- Comment être sûr qu'un traitement agit bel et bien sur la densité microfilarienne à *L. loa* et qu'il ne s'agit pas, tout simplement, d'une différence due à une variabilité aléatoire ?

- Si, pour des raisons logistiques, les prélèvements de suivi n'ont pas pu être effectués à une heure similaire à celle du prélèvement avant traitement, comment évaluer l'efficacité du traitement s'il existe une périodicité physiologique de la DMF ?

La partie 6.2 de ce document tentera de répondre à ces questions à partir des données issues d'une étude ancillaire à l'essai clinique réalisée en mars 2021.

Par ailleurs, il existe un dogme selon lequel la DMF à *L. loa* reste très stable dans le temps. Cependant, nous avons pu constater, comme décrit au chapitre 2, deux choses :

- *A priori*, si la DMF est stable dans le temps, l'évolution de celle-ci doit être proche de 0% chez les individus traités par placebo. Or, nous nous sommes rendu compte lors de l'exploitation des résultats intermédiaires que certains individus dans le groupe placebo avaient tendance à voir leur DMF augmenter au cours du temps, sur un délai assez court (moins d'un mois). Nous avons voulu déterminer si cette variabilité était due à des phénomènes aléatoires (fluctuations de la nature des flux sanguins, sur-dispersion des microfilaires dans le sang…) ou à des facteurs externes (température corporelle, température ambiante…) ou internes (périodicité de la microfilarémie due à des différences dans l'heure de prélèvement). La partie 6.3 de ce document tentera d'apporter des réponses sur cette variabilité à court terme à partir de l'exploitation de données issues de l'essai clinique.

- Certains des individus dépistés fin 2019 et classés comme pouvant être inclus dans une des cohortes de l'essai ne répondaient plus aux critères de DMF nécessaires à leur inclusion en janvier 2021. Comment expliquer ceci si la DMF à *L. loa* est stable dans le temps: heure de

prélèvement différente ? variabilité aléatoire ? La partie 6.4 s'intéressa à l'évaluation de cette variabilité à long terme à partir des données issues de l'essai clinique.

La dernière problématique d'intérêt pour ce chapitre 6 concerne également un phénomène de variabilité. Il s'agit de la variation des résultats de lecture microscopique. En effet, malgré l'expérience des deux microscopistes impliqués dans l'essai, nous avons dû faire relire des lames car nous constations des différences importantes entre les résultats des deux lecteurs. Dans n'importe quelle technique diagnostique de biologie, il existe des variations dans les résultats. En France, les comités d'accréditation des laboratoires de biologie médicale demandent la réalisation d'expériences permettant d'estimer la qualité de rendu des résultats, la variation intra- et la variation inter-lecteur. Ces expériences n'ont jamais été réalisées dans le contexte de diagnostic quantitatif de la DMF à *L. loa*. Nous avons voulu, lors du temps de terrain consacré à l'essai clinique, réaliser certaines de ces expériences afin d'évaluer cette variation.

## 6.2 Périodicité de la microfilarémie à *L. loa*

### 6.2.1 Introduction

Il est connu depuis plus de 100 ans que, chez l'homme, la DMF à *L. loa* présente une périodicité diurne, augmentant le matin pour atteindre un pic entre 10 et 15 heures, puis diminuant jusqu'à des niveaux très bas la nuit (Low 1910). La souche de *L. loa* simienne quant à elle, présente une périodicité nocturne. L'hybridation des deux souches a été réalisée expérimentalement mais, il semble qu'elle ne se produit que très rarement dans des conditions naturelles (Duke 1964). Enfin, l'heure à laquelle se produit le pic de DMF est déterminée génétiquement et correspond en réalité à l'heure d'activité maximale des vecteurs. Peu d'études se sont intéressées de manière plus approfondie aux facteurs pouvant influencer la périodicité de la DMF à *L. loa*. Parmi les quelques études sur le sujet, nous notons quelques études très anciennes datant du début ou du milieu du 20$^{ième}$ siècle. En 1921, Low & O'Driscoll ont étudié l'impact de l'inversion du cycle nycthémérale d'activité de patients (dormir la journée et travailler la nuit) sur la périodicité de *L. loa* au Nigéria. Ils réfutent la théorie de 1904 qui affirmait que

l'inversion du cycle nycthéméral des patients engendrait une inversion de la périodicité de *L. loa* de manière similaire à ce qui était observé avec *W. bancrofti* (données non publiées). En effet, ils mettent en évidence des pics de DMF entre midi et 16 heures chez des patients, quel que soit leur rythme nycthéméral d'activité (Low and O'Driscoll 1921). En 1950, Kershaw a également étudié la périodicité de la DMF à *L. loa* sur 36 heures au Cameroun chez neuf prisonniers. Bien que présentant des variations importantes dans son amplitude, l'évolution de la DMF au cours de la journée semblait similaire chez les 9 patients avec une augmentation et la diminution coïncidant avec l'aube et le crépuscule et des pics présents entre 8 heures du matin et 2 heures de l'après-midi, avec une grande proportion aux alentours de 9 heures (Kershaw 1950). En 1955, Hawking revient sur les résultats de Kershaw et réalise des études complémentaires afin de comparer l'évolution de la DMF chez des prisonniers menant une vie aux horaires très réguliers et très matinaux et chez des individus venant juste d'être hospitalisés et vivant donc selon des horaires moins réguliers et probablement moins matinaux (lever plus tard, coucher plus tard). Les pics retrouvés chez les patients nouvellement hospitalisés oscillaient entre 14 heures et 18 heures, tandis que ceux retrouvés chez les prisonniers oscillaient autour de 11 heures. Hawking conclut que cette différence de pics était probablement l'expression des habitudes diurnes moins régulières des patients nouvellement hospitalisés par comparaison aux prisonniers. De plus, la DMF à 22h chez les volontaires de l'hôpital était généralement comprise entre 0 et 20% de leur maximum de la journée, une proportion beaucoup plus élevée que celle observée chez les prisonniers à la même heure (Hawking 1955).

En 1967, Hawking *et al*. font l'hypothèse que la température corporelle et/ou ambiante peut avoir une influence sur la DMF mesurée dans le sang périphérique puisque le fait de provoquer une élévation de la température corporelle (en plaçant les sujets dans un bain chaud) déclenche une augmentation de la DMF dans les 30 minutes qui suivent (Hawking et al. 1967).

En 1976, Ogunba étudia l'évolution de la DMF chez quatre Nigérians adultes en attente d'opération chirurgicale. Des prélèvements ont été réalisés à des intervalles de deux heures pendant 14 heures. Les DMF de deux des quatre patients présentaient deux pics, l'un à 14 heures et l'autre à 18 heures, tandis que celles des deux autres patients présentait un seul pic à 18 heures. Chez les quatre patients, la DMF à 22 heures étaient toujours plus élevée qu'à 10 heures. Ogunba fait l'hypothèse d'une implication d'un autre vecteur pour *L. loa*, le moustique *Mansonia africana*, moustique très commun au Nigéria et présentant un pic d'activité de piqûre à 22 heures (Ogunba 1976).

En 1983, Carme a réévalué la périodicité de *L. loa* chez cinq sujets en République du Congo en prélevant du sang par piqûre au bout du doigt à intervalle réguliers pendant 12 heures. Ses résultats sont conformes à ceux de la littérature avec un pic de DMF à 12 heures et une évolution classique entre 6h30 et 18h00 (Carme 1983).

En 2009, Kamgno *et al*. ont caractérisé mathématiquement, pour la première fois, la périodicité des DMF à *L. loa* dans trois groupes d'individus camerounais (4 patients ayant développé un effet secondaire grave neurologique post-ivermectine, 4 patients ayant développé un effet secondaire grave non neurologique et 14 personnes témoins afin d'évaluer si les effets secondaires graves post-ivermectine pouvaient être associés à la présence d'une microfilarémie à périodicité nocturne. Aucune association n'a été mis en évidence et les pics des DMF étaient retrouvés entre 10 heures et 18 heures (Kamgno et al. 2009).

Ainsi, la littérature scientifique rapporte des évolutions de DMF à *L. loa* différentes selon les études. Les facteurs identifiés ayant un potentiel impact sur la DMF sont : le train de vie des individus (heure du lever et du coucher), la température corporelle et la période de pic optimal d'activité des vecteurs. En mars 2021, nous avons évalué la périodicité de la DMF à *L. loa* chez 13 individus vivant dans le département de la Lékoumou en République du Congo afin de tenter de répondre aux problématiques présentées en partie 6.1.

### 6.2.2 Matériels et méthodes

L'étude a été menée à Ouaka, un village forestier endémique pour la loase situé à environ 30 km de Sibiti, le chef-lieu de la Lékoumou. Treize patients ont été sélectionnés à partir d'un dépistage visant à inclure des participants dans un essai clinique visant à évaluer le lévamisole dans la prise en charge de la loase.

Chez ces 13 patients, nous avons évalué l'évolution de la DMF au cours du temps entre 9 heures et 20 heures. Des échantillons de sang ont été prélevés sur chaque individu dans un intervalle de 5 minutes autour de 9 heures, 10 heures, 11 heures, 12 heures, 13 heures, 14 heures, 15 heures, 16 heures, 18 heures et 20 heures. Des relevés de températures ambiante et corporelle ont été réalisés avant chaque prélèvement sanguin. Les DMF ont été quantifiées en utilisant des gouttes épaisses calibrées. Le sang a été prélevé par piqûre au doigt à l'aide d'une

lancette stérile et recueilli dans des tubes capillaires non héparinés. Un volume de 50 µL de sang a été étalé sur une lame, séché à température ambiante et coloré avec du colorant Giemsa dans les 24 heures suivant le prélèvement. Les microfilaires de *L. loa* ont été dénombrées au microscope en utilisant le grossissement x100 par un technicien expérimenté.

Les coefficients de corrélation de Pearson entre les variables d'intérêt (DMF, température ambiante, température corporelle et heure de prélèvements) ont été calculés et les tests de significativité ont été réalisés. Les analyses ont été réalisées en utilisant le modèle de cosinor (Nelson et al. 1979), modèle permettant d'étudier les variables régies par des rythmes circadiens. Une stratification sur le sexe et l'âge médian de la population d'étude a également été réalisée.

Le modèle de cosinor correspond à l'équation suivante : $y(t) = M + A \cos \phi \cos \omega t - A \sin \phi \sin \omega t$ où y représente la DMF observée et t représente l'heure de prélèvement. La constante $\omega = 2\pi/24$ représente la périodicité de 24 heures de la DMF de *L. loa*. Le coefficient M représente la moyenne ajustée au rythme de 24 heures définie comme la valeur moyenne de la DMF. Les paramètres A et φ, respectivement, représentent l'amplitude (définie comme la moitié de la plus grande variation de la microfilarémie au cours d'une période de 24 heures) et l'acrophase (déterminant le moment du pic) du modèle cosinor.

Enfin, le même modèle a été évalué à partir des résidus standardisés issus d'une régression linéaire mixte ajustée sur la température corporelle avec un effet aléatoire sur l'individu. Cela a permis d'évaluer, par ailleurs, l'influence possible de la température corporelle sur le niveau de DMF. Toutes les analyses ont été effectuées à l'aide du logiciel R (version 3.6.2).

### 6.2.3 Résultats

L'âge médian de la population d'étude était de 50 ans (interquartiles : 37-56) et l'âge moyen est de 47,4 ans. Parmi les 13 patients, 8 étaient des hommes et 5 étaient des femmes. Le coefficient de corrélation de Pearson entre l'heure de prélèvement et la DMF est de -0,19 ($P$ = 0,030). Celui entre la DMF et la température ambiante est de 0,11 ($P$ = 0,190). Celui entre la DMF et la température corporelle est de 0.,4 ($P$ = 0,005). Celui entre la température corporelle et l'heure de prélèvement est de -0,27 ($P$ = 0,001). Enfin, celui entre la température ambiante et la température corporelle est de 0,15 ($P$ = 0,101).

La figure 28 représente l'évolution individuelle de la DMF et de la température corporelle chez les 13 patients. Trois des patients présentent leur pic de DMF à 10 heures du matin, 2 à 11 heures, 3 à 13 heures, 2 à 14 heures, 1 à 15 heures, 1 à 16 heures et une patiente présente 2 pics identiques à 13 et 16 heures.

La figure 29 représente les DMF prédites par le modèle cosinor entre 9 heures et 20 heures pour les 13 patients stratifiés par sexe (Panel A), stratifiés sur l'âge médian (Panel B) et à partir des résidus issus d'une régression ajustée sur la température corporelle (Panel C).

Le modèle cosinor est adapté aux données (P-value du test de détection de rythme = 0.013). Le modèle prédit un pic de 4915,3 mf/mL atteint à 12h43 dans notre population d'étude, un pic de 5944,9 mf/mL atteint à 12h28 dans le groupe d'hommes, un pic de 3310,2 mf/mL atteint à 13h27 dans le groupe de femmes, un pic de 5197,0 mf/mL atteint à 12h35 dans la population des moins de 50 ans, un pic de 4676,6 mf/mL atteint à 12h50 dans la population des plus de 50 ans.

Après ajustement sur la température corporelle sur l'ensemble des 13 sujets, le pic est estimé à 4186 mf/mL à 12h02. L'amplitude diminue de manière importante (passant de 1684,8 à 310,6 mf/mL). Le test de détection de rythme est dans la limite de la significativité ($P$ = 0,066).

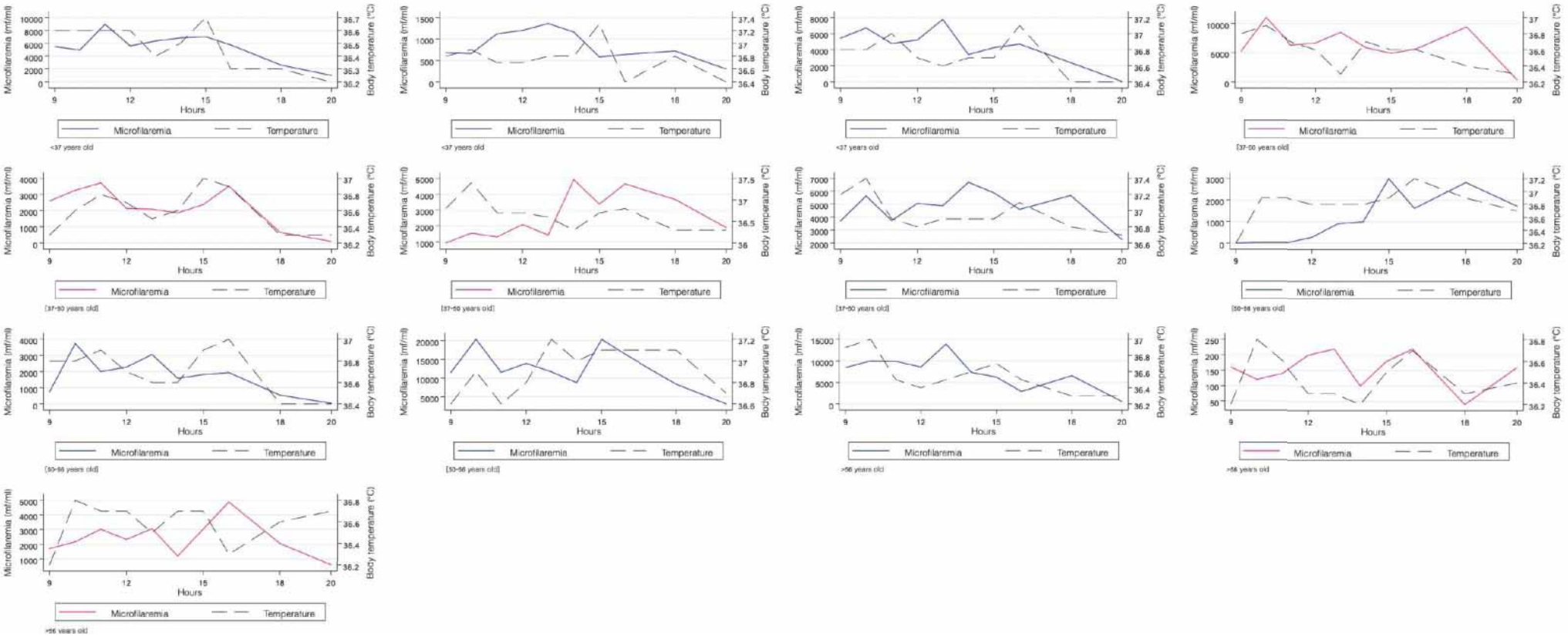


**Figure 28. Évolution individuelle de la microfilarémie à *L. loa* et de la température corporelle**

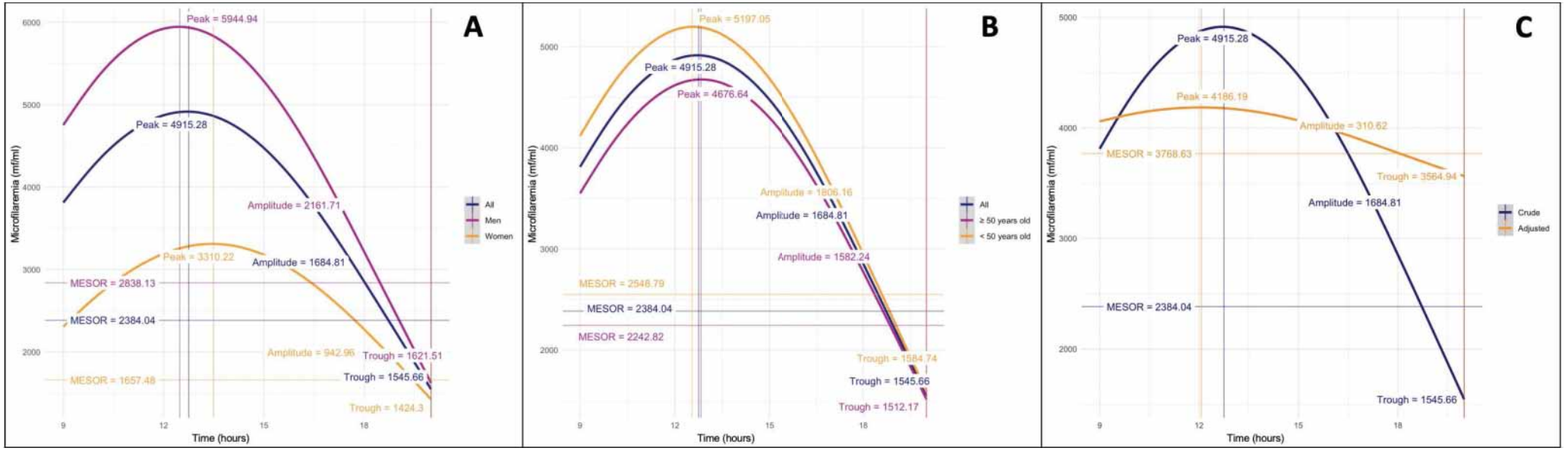


**Figure 29. Prédictions de la périodicité de la microfilarémie à *Loa loa***

(A) Modèle stratifié sur le sexe
(B) Modèle stratifié sur l'âge médian (50 ans)
(C) Modèle ajusté sur la température corporelle

### 6.2.4 Discussion

Nos résultats concordent avec ceux la littérature scientifique et notamment avec l'étude de Kamgno *et a*l. de 2009 dans laquelle le pic de DMF était estimé à 12h37 chez les personnes ayant subi un effet indésirable grave neurologique, 14h04 chez les individus ayant subi un effet indésirable grave non neurologique et 13h02 chez les individus témoins (Kamgno et al. 2009).

Nous avons estimé un horaire de pic plus précoce chez les hommes que chez les femmes mais identique chez les plus de 50 ans et les moins de 50 ans. Lorsque nous ajustons nos résultats sur la température corporelle, l'amplitude du pic est diminuée (passant de 1684 à 310) et le pic est décalé de 12h43 à 12h02. Une hypothèse serait que la température corporelle pourrait avoir une place importante dans la présence de microfilaires dans le sang mais, étant corrélé à l'heure, il reste difficile de distinguer l'influence de ces deux phénomènes. Cette hypothèse est soutenue par les travaux de Hawking (Hawking et al. 1967).

Les coefficients de corrélation de Pearson étaient significatifs entre l'heure de prélèvement et la DMF, entre la DMF et la température corporelle et entre la température corporelle et l'heure de prélèvement. Nous mettons en évidence l'existence possible d'une relation heure/température corporelle/DMF. Cependant, il est difficile de pouvoir juger de l'influence de l'heure et de la température corporelle de manière compartimentée puisqu'elles sont toutes deux corrélées également.

Néanmoins, l'examen des courbes individuelles de DMF montrent de nombreuses trajectoires différentes selon les individus dont certains présentant nettement deux pics au cours d'une journée. Ces différences pourraient être associés à des différences physiologiques des individus voire des insectes vecteurs ou également à une variabilité aléatoire dans la préparation ou l'examen microscopique des gouttes épaisses calibrées. Des études supplémentaires avec un recueil de données plus approfondi, notamment sur les habitudes de vie des individus, pourraient permettre de comprendre si la variabilité observée au niveau de la périodicité de la DMF est aléatoire ou déterminée par des facteurs externes ou internes à l'individu.

Le modèle cosinor a déjà été utilisé en parasitologie sur *Schistosoma mansoni* et *Wuchereria bancrofti* (Bogéa et al. 1996; Dixit et al. 2004). Les avantages du modèle cosinor sont de pouvoir traiter des données non équidistantes (comme c'est le cas ici) et d'être simple

d'utilisation. Le modèle cosinor possède certaines limites. Il impose de faire l'hypothèse d'une distribution normale des données, ce qui peut poser problème en parasitologie, où les données présentent souvent une forte variabilité autour de la moyenne. Les tests statistiques évaluant la qualité de l'ajustement du modèle cosinor sur nos données ne mettent cependant pas en évidence de problèmes de ce type. En outre, le modèle cosinor ne permet pas de déterminer la part de variabilité due à des facteurs externes/internes ou à une variabilité aléatoire. La limite principale du modèle cosinor est qu'il considère que chaque individu possède la même périodicité et estime des paramètres communs. Dès lors, il n'est pas possible de déterminer statistiquement si certains individus ne répondent pas à la tendance générale.

Il existe des alternatives aux modèles de cosinor permettant de s'affranchir de leurs limites et pouvant être des perspectives de recherche futures afin d'optimiser la modélisation de la périodicité de la DMF à *L. loa*. Brièvement, il est possible d'ajuster nos données selon une distribution de Von Mises, une approximation de la loi normale circulaire ou d'utiliser un processus gaussien permettant alors de déterminer un paramètre commun à la population représentant la périodicité de la DMF autour d'une valeur de base, différente entre les individus qui représenterait la charge microfilarienne de l'individu à un instant *t*.

L'utilisation du modèle cosinor ou de modèles plus complexes pourraient permettre, *in fine*, la correction des DMF dans des études longitudinales afin de diminuer les contraintes logistiques de mise en place de prélèvements à horaires réguliers au cours du suivi des populations. Les résultats obtenus ici ne permettent pas encore d'imaginer un système permettant de prédire la DMF à un instant *t* à partir d'une DMF mesurée à un instant donné chez le même patient. En effet, la variabilité dans la périodicité avec, chez certains patients, la présence de deux pics distincts reste à comprendre. La réalisation des études supplémentaires proposées dans cette discussion et l'application de modèles plus complexes pourraient permettre de mieux appréhender ces phénomènes et de les prendre en compte afin d'adapter les modèles à des facteurs spécifiques à chaque individu.

## 6.3 Variabilité à court terme de la microfilarémie à *L. loa*

### 6.3.1 Introduction

A notre connaissance, seules deux études rapportent des évaluations de microfilarémie à *L. loa* répétées quotidiennement pendant plusieurs jours sans mise en place d'une intervention dans l'intervalle. Il s'agit, pour la première, de l'étude de Low *et al*. de 1921 portant sur l'évaluation d'un traitement par injections de tartare d'antimonium sur la loase (Low and O'Driscoll 1921). Avant de mettre en place le traitement expérimental, la densité microfilarienne (DMF) à *L. loa* d'un patient a été mesurée quotidiennement à midi pendant 16 jours. Sur 16 jours, la DMF évolue entre 600 mf/mL à 2200 mf/mL selon la trajectoire suivante (figure 30). Aucun contrôle ou corrélation sur la température ambiante ou corporelle n'a été fait.

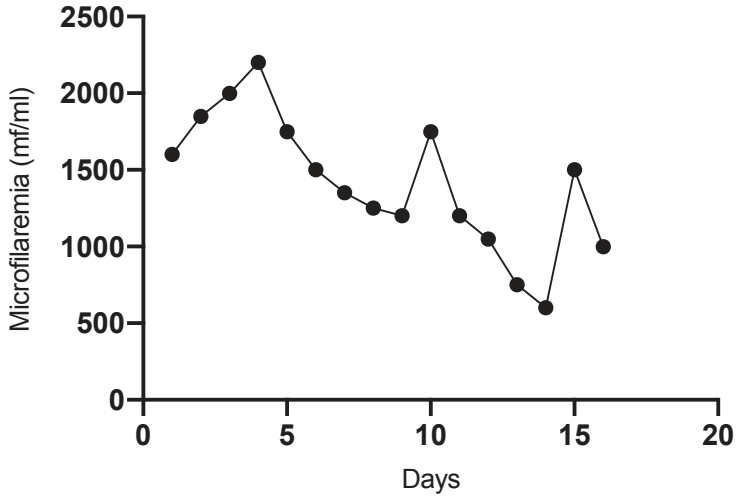


**Figure 30. Évolution individuelle de la microfilarémie chez un patient (Low et al., 1921)**

En 1950, Kershaw s'est également intéressé à la variabilité à court terme de la microfilarémie à *L. loa* au Cameroun et au Nigéria. L'évolution de la microfilarémie a été étudiée sur une période de 13 jours chez 65 prisonniers camerounais en prélevant leur sang capillaire chaque jour à midi. De plus, sur une période de 5 jours, l'évolution de la microfilarémie a aussi été évaluée chez 194 personnes vivant toutes dans un même village au Nigéria. Dans le groupe des prisonniers, 21 (32,3%) étaient microfilarémiques. Parmi eux, 66,7% présentaient une microfilarémie que l'auteur a défini comme présente en permanence dans le sang et 33,3%

présentaient une microfilarémie intermittente (c'est-à-dire qui disparaissait et réapparaissait selon les points de contrôle). Logiquement, les sujets dont les microfilaires étaient présentes de manière constante étaient ceux ayant les DMF les plus élevées. Dans le groupe de villageois, 44 (22,7%) présentaient une microfilarémie dont 39 (88,6%) une microfilarémie présente de manière constante dans le temps et 5 (11,4%) avec une microfilarémie intermittente correspondant, encore une fois, aux DMF les plus basses (Kershaw 1950). Kershaw n'a pas évalué la variabilité quantitative (quantité de microfilaires dans le sang) lors de son étude mais seulement la variabilité qualitative (présence ou absence de microfilaires dans le sang).

Nous avons réévalué la variabilité de la DMF à *L. loa* à court terme chez des individus vivant dans le département de la Lékoumou en République du Congo et pour qui plusieurs gouttes épaisses calibrées ont été réalisées au cours d'un même mois. A notre connaissance, il s'agit de la première étude évaluant la variabilité de la DMF à *L. loa* dans le temps de manière quantitative et en fonction de facteurs extérieurs tels que les températures ambiante et corporelle.

### 6.3.2 Matériels et méthodes

Dans le cadre de l'essai clinique évaluant le lévamisole sur la DMF à *L. loa*, nous avons réutilisé les données de 99 patients randomisés dans le groupe placebo et ayant été présents à tous les points de suivi. Les DMF ont été mesurées par examen de gouttes épaisses calibrés. Le sang a été prélevé par piqûre au doigt à l'aide d'une lancette stérile et recueilli dans des tubes capillaires non héparinés. Un volume de 50 µL de sang a été étalé sur une lame, séché à température ambiante, déshémoglobinisé et coloré avec du colorant de Giemsa dans les 24 heures suivant le prélèvement. Les microfilaires de *L. loa* ont été dénombrées au microscope en utilisant le grossissement x100. Chez un même patient, tous les prélèvements ont été réalisés dans la même tranche horaire, dans la mesure du possible. Des relevés de températures ambiantes moyennes quotidiennes ont été obtenus à la centrale météorologique de Sibiti, « épicentre » des villages inclus dans notre étude. Chaque lame a été lue 2 fois en aveugle par 2 microscopistes différents.

Chaque patient inclus dans cette étude a été prélevé 3 fois : une première fois puis, 5 jours et environ 1 mois (29, 30 ou 31 jours) après le premier prélèvement. Des matrices de

transitions ont été construites afin de représenter l'évolution des DMF. Une échelle semi-quantitative a été utilisée : 0-500, 501-1000, 1001-2000, 2001-5000, 5001-10 000, 10 001-20 000, et plus de 20 000 microfilaires par mL de sang.

Nous avons étudié à l'aide de plusieurs modèles de régression linéaire la relation entre (i) les différences absolues et relatives de DMF à 5 jours et 1 mois d'intervalle et (ii) le sexe, l'âge, la DMF initiale et les différences d'heure de prélèvement et de température.

Les variables dépendantes d'intérêt sont : (i) la différence relative de DMF à 5 jours d'intervalle, (ii) la différence relative de DMF à 1 mois d'intervalle, (iii) la différence absolue de DMF à 5 jours d'intervalle et (iv) la différence absolue de DMF à 1 mois d'intervalle. Pour chaque modèle, un effet aléatoire sur le village de résidence a été ajouté si sa P-value était inférieure à 0,25. Les variables explicatives intégrées dans le modèle sont : le sexe, l'âge et la DMF initiale, la différence d'heures de prélèvement et la différence de température extérieure lors des prélèvements. L'âge et la DMF initiale ont été catégorisé en fonction de leurs interquartiles. La température corporelle était relevée pour suivre l'apparition d'effets secondaires lors de l'essai clinique, et n'a donc été relevée qu'au cours de la première semaine, période où les effets secondaires sont attendus. Nous avons donc utilisé la différence de température extérieure, disponible pour tous les points de suivi à partir des relevés de la station météorologique de Sibiti. La différence relative est définie comme suit : $DMF_{final} - DMF_{initial} / DMF_{initial} \times 100$. Toutes les analyses ont été effectuées avec le logiciel STATA version 15.1.

### 6.3.3 Résultats

Le tableau 44 présente les moyennes, les médianes, les quartiles et les minimums et maximums des différences relatives et absolues à 5 jours d'intervalle et à 1 mois d'intervalle. Concernant les différences absolues : en moyenne, à 5 jours d'intervalle, les DMF ont diminué de 1033 mf/mL. Par ailleurs, 25% des individus ont diminué leur DMF d'au moins 1400 mf/mL et 25% des individus ont augmenté leur DMF d'au moins 540 mf/mL. A un mois d'intervalle, les chiffres sont similaires : en moyenne, nous constatons une diminution de 1316 mf/mL ; 25% des DMF ont diminué d'au moins 2720 mf/mL et 25% ont augmenté d'au moins 100 mf/mL.

Les tableaux 45 et 46 présentent le nombre et la proportion d'individus dont la DMF a varié de plus de 10, 20, 30, 50 et 100% par classe de DMF initiales, à 5 jours d'intervalle et 1 mois d'intervalle, respectivement. Au total, 58 sur 99 (58,8%) et 51 sur 98 (52,0%) individus n'ont pas changé de classe de DMF à 5 jours d'intervalle et 1 mois d'intervalle, respectivement (tableaux 47 et 48). Parmi ceux ayant changé de classe à 5 jours d'intervalle, 11 (26,8%) ont diminué d'une classe, 5 (12,2%) ont diminué de 2 classes, 21 (41,2%) ont augmenté d'une classe et 2 (4,9%) ont augmenté de 2 classes et 2 (4,9%) ont augmenté de 3 classes. Parmi ceux ayant changé de classe à 1 mois d'intervalle, 30 (63,8%) ont diminué d'une classe, 7 (14,9%) ont diminué de 2 classes, 1 (2,1%) a diminué de 3 classes, 9 (19,1%) ont augmenté d'une classe.

Les figures 31 et 32 représentent l'évolution de la DMF individuelle à 5 jours et à 1 mois d'intervalle, respectivement. Le coefficient de corrélation de Pearson entre les valeurs de DMF à 5 jours d'intervalle et à 1 mois d'intervalle est égale à 91,50% ($P < 0.0001$) et 91,51% ($P < 0.0001$), respectivement.

Si l'on s'intéresse à la valeur de 20 000 mf/mL, seuil à partir duquel la DMF mesurée au LoaScope contre-indique la prise d'ivermectine, on constate qu'un individu (1%) est passé d'une DMF inférieure à 20 000 (15 020 mf/mL) à une DMF supérieure à 20 000 (22 920 mf/mL) à 5 jours d'intervalle et aucun à 1 mois d'intervalle. De manière similaire, 3 individus (3%) sont passés d'une DMF supérieure à 20 000 mf/mL à une DMF inférieure à 20 000 à 5 jours d'intervalle et à 1 mois d'intervalle.

Si l'on s'intéresse à la valeur de 30 000 mf/mL, seuil à partir duquel la prise d'ivermectine est contre-indiquée par l'examen est la goutte épaisse calibrée, on constate que deux (2%) individus ont dépassé ce seuil à 5 jours d'intervalle (de 26 320 mf/mL à 32 920 mf/mL et de 29 560 mf/mL à 35360 mf/mL) et un individu a dépassé ce seuil à 1 mois d'intervalle (de 29 500 mf/mL à 38 740 mf/mL). De manière similaire, deux individus (2%) et un individu (1%) sont passés d'une DMF supérieure à 30 000 mf/mL à une DMF inférieure à 30 000 à 5 jours d'intervalle et à 1 mois d'intervalle, respectivement.

Selon la régression linéaire portant sur la différence de DMF à 5 jours d'intervalle (tableau 49), il existe un effet aléatoire à la limite de la significativité sur le village avec un ICC important, mettant en évidence un possible effet Village sur la variation de DMF.).

En raisonnant en différence relative, les individus ayant entre 1 et 999 mf/mL ont plus tendance à voir leurs DMF augmenter que les individus ayant entre 1000 et 4999 mf/mL. Cette association disparait en raisonnant en différence absolue et est remplacée par une tendance à la diminution plus importante chez ceux ayant 5000-11999 mf/mL que chez ceux ayant 1000-4999 mf/mL (non significative, P = 0.057). A 1 mois d'intervalle (tableau 50), les sujets de 59 ans et plus voient leur DMF diminuer significativement plus souvent que les 18-28 ans en différence absolue.

La température ambiante et la différence d'heure de prélèvements ne semblent pas avoir eu d'impact sur la variabilité de la DMF, que ce soit à 5 jours ou à un mois d'intervalle.

| | **5 jours d'intervalle** | | **1 mois d'intervalle** | |
|---|---|---|---|---|
| | **Différence absolue (mf/mL)** | **Différence relative (%)** | **Différence absolue (mf/mL)** | **Différence relative (%)** |
| **Moyenne ± écart-type** | – 1033 ± 5550 | + 12,6 ± 82,1 | – 1316 ± 5600 | – 8,1 ± 82,4 |
| **Médiane** | – 160 | – 5,5 | – 170 | – 23,6 |
| **10ème centile** | – 5960 | – 62,6 | – 6400 | – 70,1 |
| **1er quartile** | – 1400 | – 27,5 | – 2720 | – 48,8 |
| **3ème quartile** | + 540 | + 32,6 | + 100 | + 5,6 |
| **90ème centile** | + 2600 | + 100 | + 4820 | + 81,9 |
| **Minimum** | – 26040 | – 100 | – 31820 | – 100 |
| **Maximum** | + 18220 | + 489 | + 10200 | + 600 |

**Tableau 44. Principales mesures de la variabilité des DMF au sein de la population d'étude à 5 jours et 1 mois d'intervalle**

| Variation (N, %) | Total | 0 – 500 mf/mL | 501 – 1000 mf/mL | 1001 – 2000 mf/mL | 2001 – 5000 mf/mL | 5001 – 10 000 mf/mL | 10 001 – 20 000 mf/mL | > 20 000 mf/mL |
|---|---|---|---|---|---|---|---|---|
| **± 10%** | 80 (82,5%) | 14 (87,5%) | 12 (100%) | 7 (87,5%) | 11 (78,6%) | 14 (82,4%) | 12 (70,6%) | 10 (76,9%) |
| **± 20%** | 64 (66,0%) | 13 (81,2%) | 10 (83,3%) | 6 (75,0%) | 9 (64,3%) | 8 (47,1%) | 9 (52,9%) | 9 (69,2%) |
| **± 30%** | 49 (50,5%) | 12 (75,0%) | 10 (83,3%) | 4 (50,0%) | 5 (35,7%) | 5 (29,4%) | 7 (41,2%) | 6 (46,1%) |
| **± 50%** | 32 (33,0%) | 9 (56,3%) | 8 (66,7%) | 3 (37,5%) | 4 (28,6%) | 3 (17,7%) | 4 (23,5%) | 1 (7,7%) |
| **± 100%** | 11 (11,3%) | 7 (43,8%) | 2 (16,7%) | 1 (12,5%) | 0 (0%) | 1 (5,9%) | 0 (0%) | 0 (0%) |

**Tableau 45. Variations des microfilarémies initiales par classe de DMF initiales à 5 jours d'intervalle**

| Variation (N, %) | Total | 0 – 500 mf/mL | 501 – 1000 mf/mL | 1001 – 2000 mf/mL | 2001 – 5000 mf/mL | 5001 – 10 000 mf/mL | 10 001 – 20 000 mf/mL | > 20 000 mf/mL |
|---|---|---|---|---|---|---|---|---|
| **± 10%** | 82 (85,4%) | 16 (100%) | 8 (72,7%) | 8 (100%) | 12 (85,7%) | 13 (76,5%) | 14 (82,4%) | 11 (84,6%) |
| **± 20%** | 73 (76,0%) | 16 (100%) | 5 (45,5%) | 8 (100%) | 12 (85,7%) | 13 (76,5%) | 12 (70,6%) | 7 (53,8%) |
| **± 30%** | 56 (58,3%) | 14 (87,5%) | 3 (27,2%) | 4 (50,0%) | 10 (71,4%) | 11 (64,7%) | 8 (47,1%) | 6 (46,1%) |
| **± 50%** | 35 (36,5%) | 12 (75,0%) | 0 (0%) | 2 (25,0%) | 6 (42,9%) | 8 (47,1%) | 4 (23,5%) | 3 (23,1%) |
| **± 100%** | 11 (11,5%) | 7 (43,7%) | 0 (0%) | 0 (0%) | 1 (7,1%) | 3 (17,6%) | 0 (0%) | 0 (0%) |

**Tableau 46. Variations des microfilarémies initiales par classe de DMF initiales à 1 mois d'intervalle**

| J0 \ J5 | 0-500 | 501–1000 | 1001–2000 | 2001–5000 | 5001–10 000 | 10 001–20 000 | >20 000 | Total |
|---|---|---|---|---|---|---|---|---|
| **0–500** | 16 | 0 | 2 | 0 | 0 | 0 | 0 | 18 |
| **501–1000** | 3 | 4 | 5 | 0 | 0 | 0 | 0 | 12 |
| **501–2000** | 1 | 3 | 1 | 3 | 0 | 0 | 0 | 8 |
| **2001–5000** | 0 | 0 | 3 | 7 | 4 | 0 | 0 | 14 |
| **5001–10 000** | 0 | 1 | 0 | 4 | 11 | 1 | 0 | 17 |
| **10 001–20 000** | 0 | 0 | 0 | 1 | 6 | 9 | 1 | 17 |
| **> 20 000** | 0 | 0 | 0 | 1 | 0 | 2 | 10 | 13 |
| **Total** | 20 | 8 | 11 | 37 | 21 | 12 | 11 | 99 |

**Tableau 47. Matrice de transition de la densité microfilarienne de *L. loa* à 5 jours d'intervalle**

(colonne de gauche : résultat à J0 ; ligne du haut : résultat à J5).

| J0 \ J30 | 0-500 | 501–1000 | 1001–2000 | 2001–5000 | 5001–10 000 | 10 001–20 000 | >20 000 | Total |
|---|---|---|---|---|---|---|---|---|
| **0–500** | 17 | 1 | 0 | 0 | 0 | 0 | 0 | 18 |
| **501–1000** | 4 | 7 | 0 | 0 | 0 | 0 | 0 | 11 |
| **501–2000** | 1 | 3 | 2 | 2 | 0 | 0 | 0 | 8 |
| **2001–5000** | 0 | 3 | 5 | 4 | 2 | 0 | 0 | 14 |
| **5001–10 000** | 1 | 0 | 0 | 8 | 4 | 4 | 0 | 17 |
| **10 001–20 000** | 0 | 0 | 0 | 1 | 9 | 7 | 0 | 17 |
| **> 20 000** | 0 | 0 | 0 | 0 | 2 | 1 | 10 | 13 |
| **Total** | 23 | 14 | 7 | 15 | 17 | 12 | 10 | 98 |

**Tableau 48. Matrice de transition de la densité microfilarienne de *L. loa* à un mois d'intervalle**

(colonne de gauche : résultat à J0 ; ligne du haut : résultat à J5).

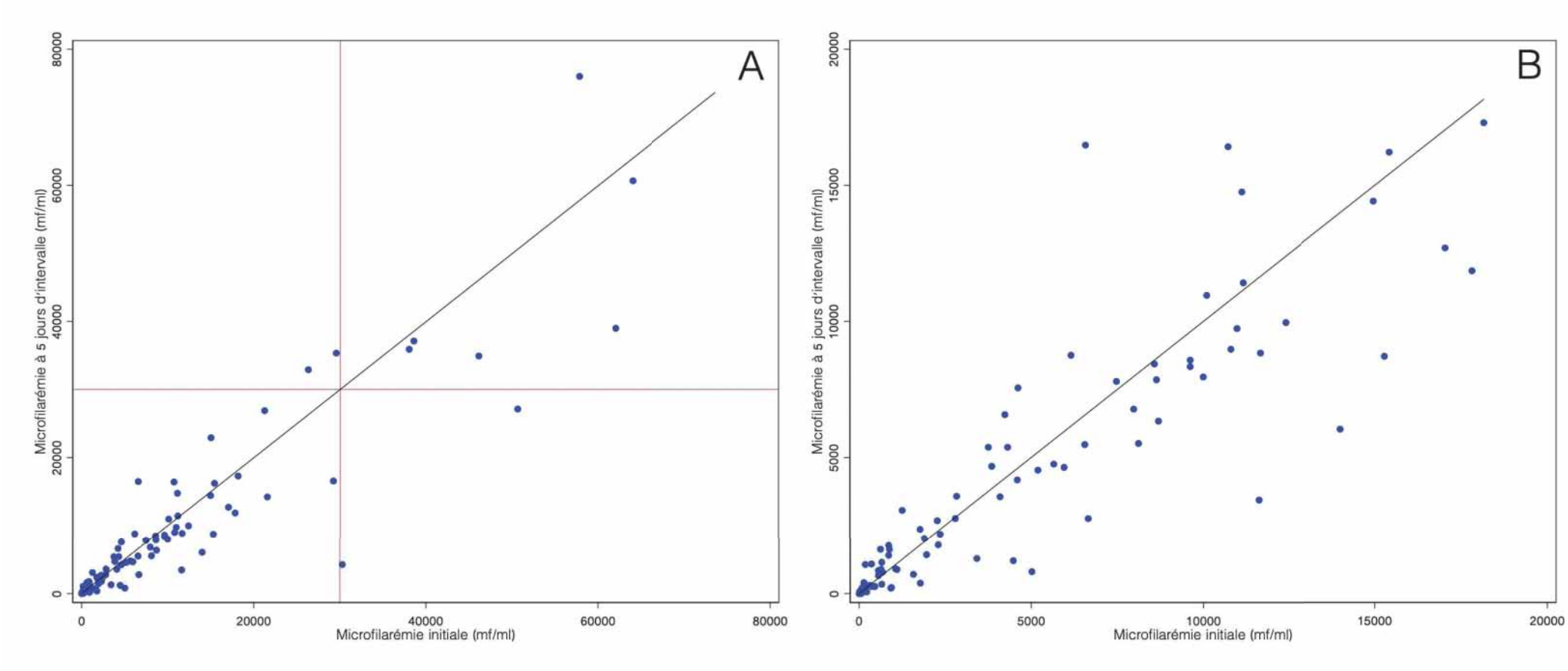


**Figure 31. Densités microfilariennes initiales et à 5 jours d'intervalle**

(A) Tous les individus

(B) Focus sur les DMF inférieures à 20 000 mf/mL

La ligne oblique noire représente DMF initiale = DMF à 5 jours

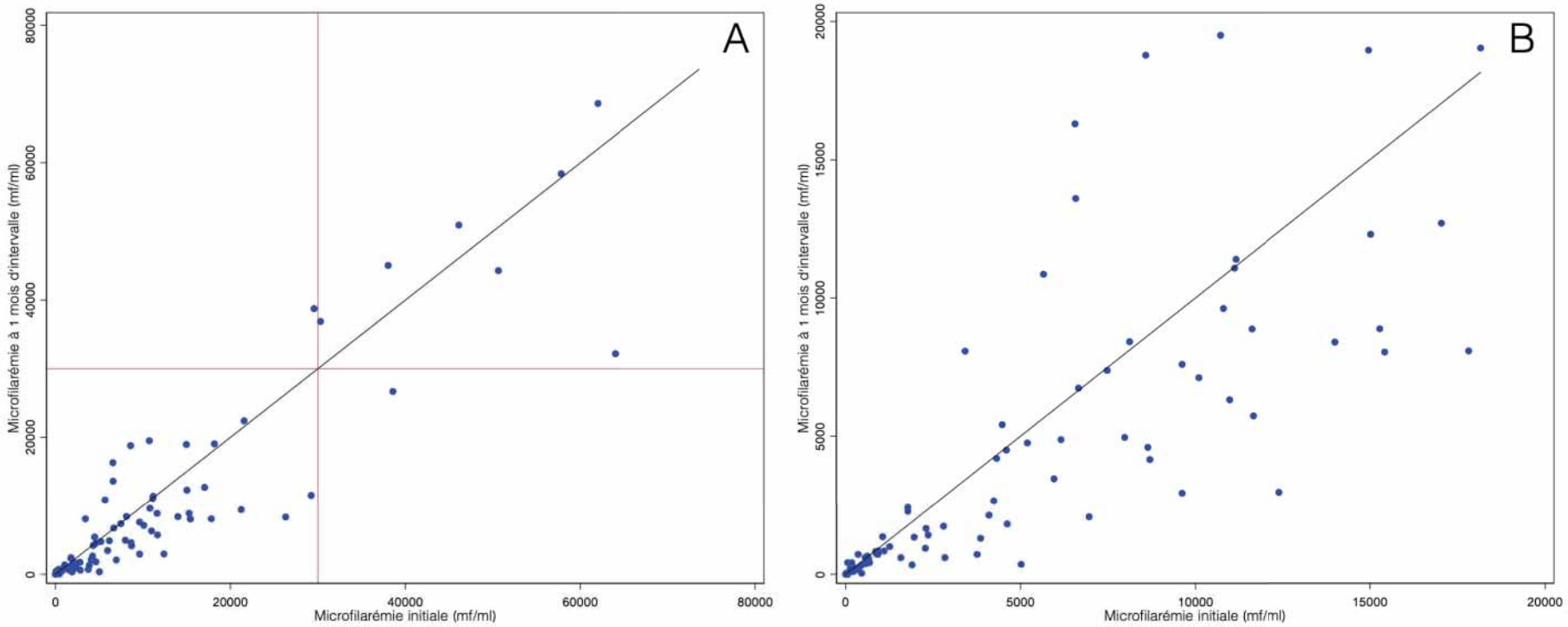


**Figure 32. Densités microfilariennes initiales et à 1 mois d'intervalle**

(A) Tous les individus

(B) Focus sur les DMF inférieures à 20 000 mf/mL

La ligne oblique noire représente DMF initiale = DMF à 1 mois d'intervalle

| | *Différence relative* | | | *Différence absolue* | | |
|---|---|---|---|---|---|---|
| | Coeff. | 95% CI | *P* | Coeff. | 95% CI | *P* |
| **Sexe** | | | | | | |
| Femme | Ref. | | | Ref. | | |
| Homme | + 18,9% | - 16,2 – + 54,1% | .979 | - 1831 | - 4239 – + 577 | .136 |
| **Age** | | | | | | |
| 18–28 ans | - 0,7% | - 51,7 – +50,4% | .979 | + 984 | - 2605 – + 4574 | .591 |
| 29–38 ans | Ref. | | | Ref. | | |
| 39–48 ans | + 1,4% | - 48,6 – + 51,4% | .955 | + 2339 | - 1178 – + 5857 | .192 |
| 49–58 ans | - 13,8% | - 56,2 – + 28,6% | .523 | + 1689 | - 1259 – + 4638 | .262 |
| 59 ans et + | - 18,4% | - 63,7 – + 27,0% | .427 | - 352 | - 3463 – + 2759 | .825 |
| **Microfilarémie initiale** | | | | | | |
| 1–999 mf/mL | + 45,6% | + 8,1 – + 83,0% | .017 | + 738 | - 1804 – + 3280 | .569 |
| 1000–4999 mf/mL | Ref. | | | | | |
| 5000–11 999 mf/mL | - 38,0% | - 81,3 – + 5,4% | .086 | - 3333 | - 6342 – - 323 | .030 |
| 12 000 mf/mL et + | + 17,9% | - 30,0 – + 65,8% | .463 | - 564 | - 3882 – + 2753 | .739 |
| **Différence de temps de prélèvement** | | | | | | |
| < – 15 min | + 20,9% | - 42,3 – + 84,2% | .517 | - 493 | - 4961 – + 3973 | .828 |
| – 15 min à +15 min | Ref. | | | | | |
| > + 15 min | - 3,5% | - 53,8 – + 46,7% | .890 | - 1182 | - 3603 – + 2238 | .498 |
| **Différence de température extérieure** | | | | | | |
| < – 2°C | + 12,9% | - 20,6 – + 46,3% | .451 | + 820 | - 1437 – + 3077 | .476 |
| – 2°C à + 2°C | Ref. | | | | | |
| > + 2°C | - 21,1% | - 86,3 – + 44,1% | .526 | - 2525 | - 6986 – + 1937 | .267 |
| **Effet aléatoire sur le village de résidence** | Coefficient de corrélation intra-classe = 25,1% (*P* = .044) | | | Coefficient de corrélation intra-classe = 7,1% (*P* = .107) | | |

**Tableau 49. Résultats de la régression linéaire sur la différence relative et absolue à 5 jours d'intervalle**

Pour les différences de température et d'heure de prélèvements, comprendre $X_{initial} - X_{final}$

| | *Différence relative* | | | *Différence absolue* | | |
|---|---|---|---|---|---|---|
| | Coeff. | 95% CI | *P* | Coeff. | 95% CI | *P* |
| **Sexe** | | | | | | |
| Femme | Ref. | | | Ref. | | |
| Homme | - 17,9% | - 55,8 – + 20,0% | .354 | + 247 | - 2073 – + 2568 | .835 |
| **Age** | | | | | | |
| 18–28 ans | - 6,1% | - 65,0 – + 52,8% | .839 | - 482 | - 4092 – + 3127 | .793 |
| 29–38 ans | Ref. | | | Ref. | | |
| 39–48 ans | + 4,3% | - 47,4 – + 56,1% | .869 | - 519 | - 3690 – + 2651 | .748 |
| 49–58 ans | - 4,5% | - 51,4 – + 42,3% | .849 | - 759 | - 3618 – + 2100 | .603 |
| 59 ans et + | - 1,6% | - 50,5 – + 47,3% | .948 | - 3054 | - 6045 – - 62 | .045 |
| **Microfilarémie initiale** | | | | | | |
| 1–999 mf/mL | + 12,5% | - 27,9 – + 52,9% | .544 | + 861 | - 1592 – + 3315 | .491 |
| 1000–4999 mf/mL | Ref. | | | | | |
| 5000–11 999 mf/mL | + 3,5 % | - 45,8 – + 52,9% | .899 | - 2410 | - 5435 – + 614 | .118 |
| 12 000 mf/mL et + | - 7,1% | - 65,1 – + 50,9% | .811 | - 3256 | - 6813 – + 301 | .073 |
| **Différence de temps de prélèvement** | | | | | | |
| < – 15 min | - 7,0 % | - 77,8 – + 63,5% | .846 | - 3507 | - 7843 – + 829 | .113 |
| – 15 min à +15 min | Ref. | | | | | |
| > + 15 min | - 45,4 % | - 121,7 – + 31,0% | .244 | - 1414 | - 6093 – + 3264 | .553 |
| **Différence de température extérieure** | | | | | | |
| *Missing Data* | + 42,3 % | - 8,2 – + 92,8% | .100 | + 4390 | + 1296 – + 7484 | .005 |
| – 2°C à + 2°C | Ref. | | | | | |
| > + 2°C | - 31,7% | - 154,9 – + 91,4% | .614 | - 3227 | - 10779 – + 4325 | .402 |
| **Effet aléatoire sur le village de résidence** | Coefficient de corrélation intra-classe non significatif (*P* = 1.000) | | | Coefficient de corrélation intra-classe non significatif (*P* = 1.000) | | |

**Tableau 50. Résultats de la régression linéaire sur la différence relative et absolue à 1 mois d'intervalle**

Pour les différences de température et d'heure de prélèvements, comprendre $X_{final} – X_{initial}$

### 6.3.4 Discussion

Au niveau collectif, à 5 jours et 1 mois d'intervalle, la DMF reste constante dans le temps (coefficients de corrélation très significatifs de 91,50 et 91,51%, respectivement). Néanmoins lorsqu'on examine les résultats au niveau individuel, on constate de grandes variations chez certains individus : 25% de la population a vu sa DMF diminuer d'au moins 1400 mf/mL en 5 jours et de 2720 mf/mL en 1 mois. Lorsqu'on s'intéresse aux pourcentages de variation, plus de 50% de la population a connu une variation de ±30% à 5 jours et 1 mois d'intervalle. Bien qu'essentiellement observées chez des sujets ayant de faibles DMF (qui engendrent logiquement des pourcentages de variations plus importants), nous retrouvons de grandes variations allant jusqu'à ± 50% dans les classes de haute DMF.

Lors de l'essai, chaque patient avait une carte de participant nous permettant de l'identifier et ainsi de limiter les erreurs d'identitovigilance.

On constate qu'un seul individu (1%) a dépassé le seuil de 20 000 mf/mL à 5 jours d'intervalle. Si l'on considère notre population d'étude de 98 personnes, ce résultat pourrait avoir des impacts pour les campagnes de traitement à venir, car il peut signifier qu'il est primordial de tester la DMF au plus près de la prise d'ivermectine si 1% de la population sa DMF varier à court terme.

Nous ne mettons pas en évidence de facteurs extérieurs (température ou heure de prélèvements) ayant un impact sur la variabilité de la DMF à l'aide des régressions linéaires. La température n'a que peu varié au cours de l'étude (de -3,1°C à +2,9°C au maximum de différence) et les prélèvements ont été effectués à horaires assez réguliers (plus de 90% des prélèvements avaient une différence de temps comprise entre -11 minutes et +15 minutes). Des différences plus importantes auraient probablement eu un impact sur nos résultats. Enfin, un effet aléatoire sur le village améliore les modèles à 5 jours mais pas à 1 mois. Cela est difficile à expliquer mais pourrait provenir d'un manque de puissance ou de facteurs de confusion extérieurs non connus.

Nos résultats ont des limites. En effet, la variabilité observée peut être en partie due à la variabilité induite par les opérateurs lors de la quantification des microfilaires par l'examen du prélèvement au microscope. De plus, même si un recueil des traitements était réalisé à chaque point de mesure, un biais de mémorisation sur la prise d'un traitement pourrait exister même si cela est peu probable sur une si petite période de temps.

Nos résultats mettent en évidence l'existence d'une forte stabilité dans le temps de la DMF au niveau collectif. Toutefois, nous mettons également en évidence une variabilité individuelle importante chez certains individus. Cette variabilité pourrait potentiellement influencer les résultats d'études longitudinales ayant comme critère de décision la différence absolue ou relative de DMF à *L. loa* après application d'une intervention. Nous attirons également l'attention sur l'existence de quelques individus ayant dépassé le seuil de 20 000 ou 30 000 mf/mL, pouvant avoir des conséquences pour les programmes de traitements à large échelle.

## 6.4 Variabilité à long terme de la microfilarémie à *L. loa*

### 6.4.1 Introduction

Plusieurs études décrivent la DMF à *L. loa* comme très stable dans le temps si aucun traitement n'est administré. La première étude sur la variabilité à long terme de la DMF à *L. loa* date de 1992 et a été menée dans le département de la Lékoumou, au Congo-Brazzaville (Noireau and Pichon 1992). Cent-quatre-vingt-douze individus ont subi un dépistage par goutte épaisse calibrée en février 1985. Deux mois plus tard, 171 de ces individus ont été retrouvés et réexaminés. Enfin, 3 ans plus tard, 66 de ces individus ont été examinés une troisième fois. Aucun individu n'a rapporté avoir pris un traitement au cours de l'étude. Au niveau communautaire, la DMF est resté stable au cours du temps chez les 42 individus microfilarémiques retrouvés à deux mois (coefficient de corrélation = 0,94, $P < 0,001$) avec une moyenne initiale de de 3,3 mf/40mm$^3$, passée à 3,2 mf/40mm$^3$, deux mois après.

Chez les 28 individus microfilarémiques retrouvés à trois ans, le coefficient de corrélation entre les DMF était de 0,79 ($P < 0,001$) avec une moyenne initiale de de 3,7 mf/40mm$^3$, passée à 3,8 mf/40mm$^3$ trois ans plus tard.

Une deuxième étude ayant évalué la stabilité dans le temps a été publiée en 1995 (Garcia et al. 1995). Sur une période d'un an, les auteurs ont prélevé 667 individus tous les deux mois. Au final, 195 sujets microfilarémiques ont été prélevés au moins 2 fois (les prélèvements manquants étaient des perdus de vus). Aux niveaux communautaire et individuel, les auteurs concluent que la prévalence de la microfilarémie et la DMF se sont révélées très stables dans le temps.

En 2019, l'étude de Pion SDS *et al*. s'est intéressée à l'évolution de la DMF à 18 mois d'intervalle chez des patients traités par ivermectine et d'autres exclus du traitement de masse pour contre-indication (DMF à *L. loa* trop élevée, grossesse ou maladie aiguë). Les auteurs rapportent que 70% des 291 patients non traités, toutes causes confondues, sont restés dans la même classe de DMF 18 mois plus tard mais également que 38,1% des 154 non traités pour DMF trop élevées ont vu leur DMF diminuer en dessous du seuil de risque d'apparition d'effets indésirables graves post-ivermectine.

A notre connaissance, seules ces 3 études ont évalué la variabilité de la DMF à *L. loa* à long terme. Les auteurs de ces 3 études n'indiquent pas s'ils ont tenté de prélever les patients à la même heure à chaque point de mesure ou s'ils ont ajusté sur l'heure de prélèvement. Enfin, aucune de ces études ne s'est intéressée à l'impact potentiel de la température ambiante sur les variations de DMF. De plus, il ne semble pas exister de consensus définissant la « stabilité » de la DMF au cours du temps.

Lors de notre essai clinique évaluant le lévamisole, nous nous sommes rendu compte que certains individus avaient, début 2021, une DMF qui avait varié considérablement depuis leur premier dépistage fin 2019. Ainsi, nous avons réalisé une étude visant à évaluer la variabilité de la DMF chez des individus examinés deux fois à 14 mois d'intervalle. Nous avons enregistré les heures de prélèvement et les températures ambiantes afin de déterminer, pour la première fois, si ces facteurs extérieurs ont un impact sur la stabilité de la DMF.

### 6.4.2 Matériels et méthodes

Les données du dépistage réalisé avant traitement en 2021 ont été combinées avec celles du dépistage réalisé en octobre 2019 afin d'identifier le site d'étude pour l'essai clinique. Un volume de 50 µL de sang a été étalé sur une lame, séché à température ambiante, déshémoglobinisé et coloré avec du colorant de Giemsa dans les 24 heures suivant le prélèvement. Les microfilaires de *L. loa* ont été dénombrées au microscope en utilisant le grossissement x100. Les lames ont été lues une fois par un technicien expérimenté. Les heures de prélèvement ont été enregistrées et catégorisées en « - de 30 minutes de différence par rapport à 2019 » et « + de 30 de minutes de différence par rapport à 2019 ». Les villages de résidence ont été catégorisés en fonction du nombre de villages les séparant d'un point où les campagnes d'administration de masse par ivermectine sont menées.

Des matrices de transitionsont été construites pour représenter l'évolution des DMF sur une échelle semi-quantitative dans les groupes suivants : 0-500, 501-1000, 1001-2000, 2001-5000, 5001-10 000, 10 001-20 000, et plus de 20 000 microfilaires par mL de sang.

Enfin, afin d'étudier (i) la différence relative de DMF et (ii) la différence absolue de DMF, deux modèles de régression linéaire ont été réalisés.

La différence relative est définie comme suit : $DMF_{2021} – DMF_{2019} / DMF_{2019} \times 100$. Toutes les analyses ont été effectuées avec le logiciel STATA version 15.1.

### 6.4.3 Résultats

Au total, 259 individus ont été inclus dans cette étude. Chaque individu a subi un dépistage en octobre 2019 et au début de 2021 (janvier-mars). En 2019, la DMF moyenne était de 8528 ± 14 723 mf/mL. En 2021, la DMF moyenne était de 6809 ± 11 493 mf/mL. Le coefficient de corrélation de Pearson entre les DMF de 2019 et 2021 était de 80,0% ($P < 0,0001$).

Le tableau 51 présente les moyennes, les médianes, les quartiles et les minimums et maximums des différences relatives et absolues des DMF mesurées en 2019 et 2021. En raisonnant en différence absolue, en moyenne, les DMF ont diminué de 1718 mf/mL à deux ans d'intervalle. Par ailleurs, 25% des individus ont vu leur DMF diminuer d'au moins 2980 mf/mL et 25% des individus l'ont vu augmenter d'au moins 760 mf/mL.

Le tableau 52 présente le nombre et la proportion d'individus pour lesquels la DMF a varié de plus de 10, 20, 30, 50 et 100%, ceci par classe de DMF initiales. Le tableau 53 présente la même chose mais uniquement pour individus dont les deux prélèvements ont été réalisés à moins de 30 minutes d'intervalle.

Seulement 113 individus (43,6%) n'ont pas changé de classe de DMF entre 2019 et 2021 (tableau 54). Parmi ceux ayant changé de classe, 55 (37,7%) ont diminué d'une classe, 22 (15,1%) ont diminué de 2 classes, 17 (11,6%) ont diminué de 3 classes ou plus, 37 (25,3%) ont augmenté d'une classe et 15 (10,3%) ont augmenté de 2 classes. Un seul individu a dépassé les 20 000 mf/mL en 2021 alors qu'il était en dessous en 2019 et aucun n'a dépassé les 30 0000 mf/mL.

La différence entre les heures de prélèvement en 2019 et 2021 était inférieure à 30 minutes chez 58 sujets. Parmi ceux-ci, 34 (58,6%) sont restés dans la même classe de DMF entre 2019 et 2021 (tableau 55) et aucun n'a dépassé les 20 000 mf/mL ou 30 000 mf/mL.

| | Entre 2019 et 2021 | |
|---|---|---|
| | **Différence absolue (mf/mL)** | **Différence relative (%)** |
| **Moyenne ± écart-type** | – 1718 ± 9218 | + 42,5 ± 237,5 |
| **Médiane** | – 600 | – 29,9 |
| **10ème centile** | – 7260 | – 95,7 |
| **1er quartile** | – 2980 | – 71,0 |
| **3ème quartile** | + 760 | + 50,0 |
| **90ème centile** | + 5620 | + 248 |
| **Minimum** | – 36100 | – 100 |
| **Maximum** | + 18220 | + 768 |

**Tableau 51. Principales mesures de la variabilité de la DMF entre 2019 et 2021**

La figure 33 montre l'évolution de la DMF individuelle entre 2019 et 2021. Le tableau 56 présente les résultats des analyses de régression linéaire sur la différence relative et la différence absolue entre les DMF mesurées en 2019 et en 2021. Si l'on considère les différences relatives, les DMF augmentent de manière plus marquée chez les hommes que chez les femmes ; ce résultat disparait si l'on considère les différences absolues. De même, les individus ayant les DMF les plus élevées en 2019 ont tendance à voir leurs DMF augmenter de façon plus marquée que les individus ayant des DMF faibles, que ce soit en différence relative ou absolue. L'âge, la proximité des points de distribution d'ivermectine, les différences d'heure de prélèvement et les différences de température ne semblent pas avoir d'impact sur la différence relative ou absolue entre les DMF mesurées à 14 mois d'intervalle.

| Variation (N, %) | Total | 0 – 500 mf/mL | 501 – 1000 mf/mL | 1001 – 2000 mf/mL | 2001 – 5000 mf/mL | 5001 – 10 000 mf/mL | 10 001 – 20 000 mf/mL | > 20 000 mf/mL |
|---|---|---|---|---|---|---|---|---|
| **± 10%** | 239 (93,0%) | 30 (90,9%) | 37 (97,4%) | 41 (97,6%) | 42 (93,3%) | 36 (97,3%) | 29 (87,9%) | 24 (82,8%) |
| **± 20%** | 221 (86,0%) | 29 (87,9%) | 35 (92,1%) | 40 (95,2%) | 41 (91,1%) | 33 (89,2%) | 24 (72,7%) | 19 (65,5%) |
| **± 30%** | 204 (79,4%) | 27 (81,8%) | 35 (92,1%) | 38 (90,5%) | 38 (84,4%) | 29 (78,4%) | 19 (57,6%) | 18 (62,1%) |
| **± 50%** | 161 (62,6%) | 23 (69,7%) | 29 (76,3%) | 33 (78,6%) | 34 (75,6%) | 17 (45,9%) | 13 (39,4%) | 12 (41,4%) |
| **± 100%** | 60 (23,4%) | 19 (39,4%) | 16 (42,1%) | 14 (33,3%) | 10 (22,2%) | 3 (8,1%) | 2 (6,1%) | 2 (6,9%) |

**Tableau 52. Variations des DMF entre 2019 et 2021 par classe de densités initiales**

| Variation (N, %) | Total | 0 – 500 mf/mL | 501 – 1000 mf/mL | 1001 – 2000 mf/mL | 2001 – 5000 mf/mL | 5001 – 10 000 mf/mL | 10 001 – 20 000 mf/mL | > 20 000 mf/mL |
|---|---|---|---|---|---|---|---|---|
| **± 10%** | 55 (94,8%) | 7 (100%) | 6 (100%) | 9 (100%) | 8 (80,0%) | 12 (100%) | 4 (100%) | 9 (90,0%) |
| **± 20%** | 53 (91,4%) | 7 (100%) | 6 (100%) | 9 (100%) | 8 (80,0%) | 12 (100%) | 4 (100%) | 7 (70,0%) |
| **± 30%** | 46 (79,3%) | 6 (85,7%) | 6 (100%) | 7 (77,8%) | 8 (70,0%) | 10 (83,3%) | 2 (75,0%) | 7 (70,0%) |
| **± 50%** | 31 (53,4%) | 6 (85,7%) | 5 (83,3%) | 4 (44,4%) | 6 (60,0%) | 6 (50,0%) | 1 (25,0%) | 3 (30,0%) |
| **± 100%** | 11 (19,0%) | 4 (57,1%) | 1 (16,7%) | 1 (11,1%) | 2 (20,0%) | 3 (25,0%) | 0 (0%) | 0 (0%) |

**Tableau 53. Variations des DMF entre 2019 et 2021 par classe de densités initiales chez les patients ayant des heures de prélèvement similaires (± 30 minutes)**

| 2019 \ 2021 | 0-500 | 501–1000 | 1001–2000 | 2001–5000 | 5001–10 000 | 10 001–20 000 | >20 000 | Total |
|---|---|---|---|---|---|---|---|---|
| **0–500** | 22 | 7 | 4 | 2 | 0 | 0 | 0 | 35 |
| **501–1000** | 17 | 6 | 6 | 9 | 0 | 0 | 0 | 38 |
| **501–2000** | 11 | 9 | 4 | 18 | 0 | 0 | 0 | 42 |
| **2001–5000** | 9 | 5 | 10 | 21 | 0 | 0 | 0 | 45 |
| **5001–10 000** | 2 | 1 | 2 | 0 | 27 | 5 | 0 | 37 |
| **10 001–20 000** | 1 | 1 | 1 | 0 | 13 | 16 | 1 | 33 |
| **> 20 000** | 2 | 0 | 0 | 0 | 4 | 6 | 17 | 29 |
| **Total** | 64 | 29 | 27 | 50 | 44 | 27 | 18 | 259 |

**Tableau 54. Matrice de transition de la densité microfilarienne de *L. loa* en 2019 par rapport à celle de 2021**

| 2019 \ 2021 | 0-500 | 501–1000 | 1001–2000 | 2001–5000 | 5001–10 000 | 10 001–20 000 | >20 000 | Total |
|---|---|---|---|---|---|---|---|---|
| **0–500** | 2 | 1 | 2 | 2 | 0 | 0 | 0 | 7 |
| **501–1000** | 3 | 0 | 3 | 0 | 0 | 0 | 0 | 6 |
| **501–2000** | 2 | 3 | 1 | 2 | 0 | 0 | 0 | 9 |
| **2001–5000** | 1 | 0 | 2 | 17 | 0 | 0 | 0 | 10 |
| **5001–10 000** | 0 | 0 | 1 | 0 | 7 | 4 | 0 | 12 |
| **10 001–20 000** | 0 | 0 | 0 | 0 | 3 | 1 | 0 | 4 |
| **> 20 000** | 0 | 0 | 0 | 0 | 0 | 4 | 6 | 10 |
| **Total** | 8 | 4 | 9 | 12 | 10 | 9 | 6 | 58 |

**Tableau 55. Matrice de transition de la densité microfilarienne de *L. loa* en 2019 par rapport à celle de 2021 seulement chez les individus ayant eu leurs deux prélèvements faits à moins de 30 minutes d'intervalle.**

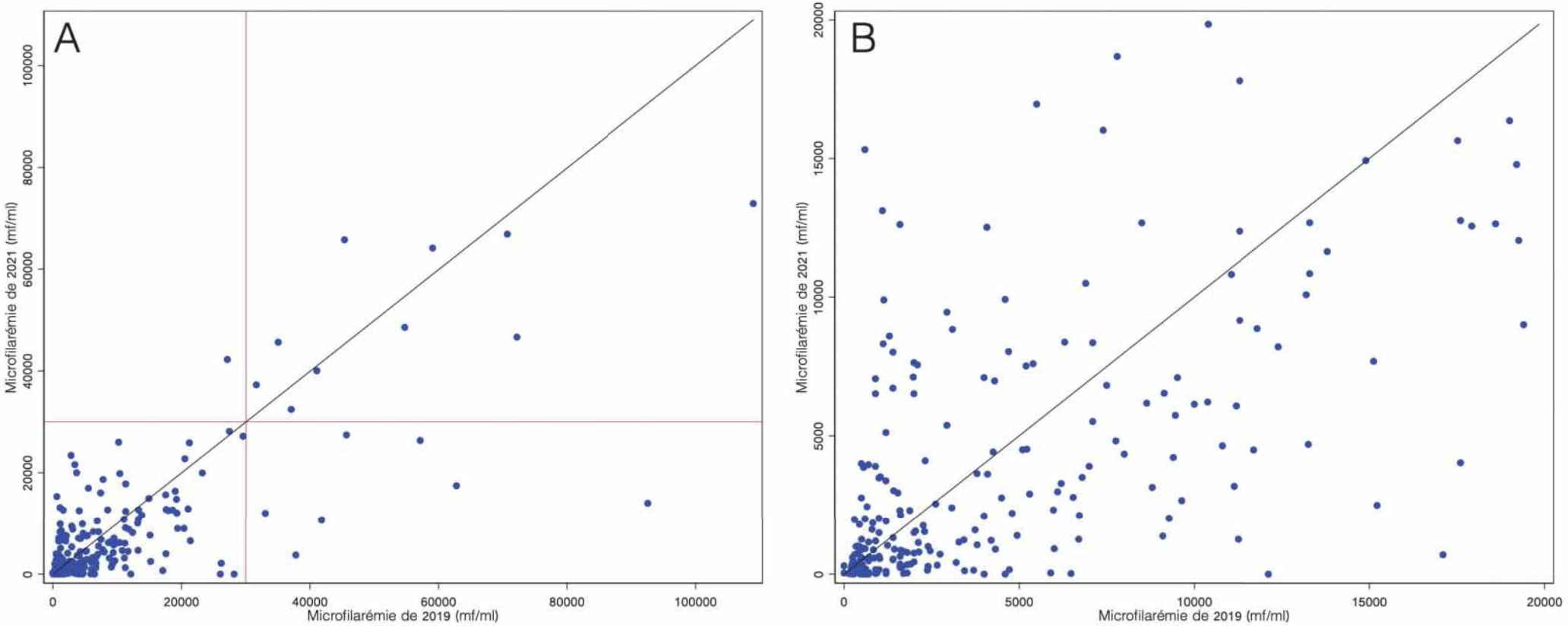


**Figure 33. Densités microfilariennes en 2019 et en 2021**

(A) Tous les individus

(B) Focus sur les DMF inférieures à 20 000 mf/mL

La ligne oblique noire représente DMF de 2019 = DMF de 2021

| | *Différence relative* | | | *Différence absolue* | | |
|---|---|---|---|---|---|---|
| | Coeff. | 95% CI | *P* | Coeff. | 95% CI | *P* |
| **Sexe** | | | | | | |
| Femme | Ref. | | | Ref. | | |
| Homme | + 46,7% | + 16,4 – + 77,0% | .003 | + 1352 | - 874 – + 3579 | .233 |
| **Age** | | | | | | |
| 18–28 ans | - 19,2% | - 69,7– + 31,2% | .453 | - 1731 | - 5440 – + 1977 | .359 |
| 29–38 ans | Ref. | | | Ref. | | |
| 39–48 ans | - 14,9% | - 61,2 – + 31,3% | .526 | - 528 | - 3930 – + 2872 | .760 |
| 49–58 ans | - 27,8% | - 69,3 – + 13,7% | .189 | - 2245 | - 5299 – + 807 | .149 |
| 59 ans et + | + 1,3% | - 45,2 – + 47,8% | .956 | - 1781 | - 5203 – + 1640 | .306 |
| **Microfilarémie initiale** | | | | | | |
| 1–999 mf/mL | + 7,4% | - 30,2 – + 45,0% | .698 | + 69 | - 2696 – + 2834 | .961 |
| 1000–4999 mf/mL | Ref. | | | Ref. | | |
| 5000–11 999 mf/mL | - 23,7% | - 64,4 – + 17,0% | .253 | - 1565 | - 4559 – + 1428 | .304 |
| 12 000 mf/mL et + | - 40,1% | - 80,4 – + 0,2% | .051 | - 10371 | - 13336 – - 7406 | .001 |
| **Proximité de l'ivermectine** | | | | | | |
| Proche | - 17,5% | - 51,6 - + 16,7% | .314 | + 461 | - 2048 – + 2972 | .717 |
| Moyennement proche | - 8,8% | - 45,4 - + 27,9% | .637 | - 645 | - 3341 – +2049 | .637 |
| Éloigne | Ref. | | | Ref. | | |
| **Différence de temps de prélèvement** | | | | | | |
| - de 30 min de différence | Ref. | | | Ref. | | |
| + de 30 min de différence | - 0,6% | - 34,1 – + 32,9% | .973 | + 1360 | - 1104 – + 3825 | .278 |
| **Différence de température** | | | | | | |
| < –2°C | + 17,8% | - 31,3 – +67,0% | .476 | - 639 | - 4254 – +2976 | .728 |
| Entre – 2°C et +2°C | Ref. | | | Ref. | | |
| > + 2°C | - 2,0% | - 40,6 – +36,6% | .919 | - 589 | - 3245 – +2246 | .682 |

**Tableau 56. Résultats de la régression linéaire sur les différences relative et absolue des DMF à 1 an d'intervalle**

Pour les différences de température et d'heure de prélèvements, comprendre $X_{final} - X_{initial}$

### 6.4.4 Discussion

De manière similaire à la variabilité à court terme, les DMF mesurées à 1 an d'intervalle sont très stables au niveau collectif. Au niveau individuel, des variations importantes ont été observées, allant jusqu'à ±100% chez certains individus. Les hautes variations sont essentiellement retrouvées chez les sujets ayant de faibles DMF, du fait de l'artéfact de présenter les résultats en pourcentage. Néanmoins, nous mettons en évidence des variations pouvant aller jusqu'à ± 50% dans tous les classes de DMF, même les plus élevées.

Il est possible que des biais de confusion (biais de mémorisation, biais d'identification…) soient à l'origine de ces différences. Néanmoins, il est également possible que ces différences soient dues, au moins en partie, à une variabilité de la qualité de la lecture des gouttes épaisses entre 2019 et 2021. La dernière partie de ces travaux de thèse va s'intéresser à quantifier cette variabilité.

## 6.5 Variabilité dans le diagnostic quantitatif de la loase

### Premiers éléments requis dans un objectif d'accréditation de la méthode microscopique actuelle pour le diagnostic quantitatif de la loase

*Non publié*

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#### 6.5.1 Introduction

Le diagnostic de la loase repose sur l'observation des microfilaires dans le sang périphérique par examen au microscope optique d'une goutte épaisse calibrée. En pratique, après piqûre au bout doigt du patient, un volume standardisé (généralement 50 µL) de sang est collecte à l'aide d'un capillaire à hématocrite, puis déposé et étalé sur une lame porte-objet. La lame est laissée à sécher à température ambiante, puis colorée au colorant Giemsa. Le technicien lit ensuite l'ensemble de la lame en comptant les microfilaires et multiplie ensuite la quantité trouvée pour la rapporter au millilitre de sang (mf/mL). Bien que cette méthode diagnostique soit largement utilisée en Afrique centrale pour le diagnostic individuel ou pour les campagnes de dépistage de masse et qu'elle soit recommandée par la France (Comede 2015), l'Australie (Chaves et al. 2016) et l'Angleterre (Checkley et al. 2010) pour la prise en charge des patients revenant de zones endémiques, elle n'a jamais fait l'objet d'une validation ou d'une accréditation. En 2015, l'agence nationale de sécurité du médicament (ANSM) a publié un rapport national de contrôle de qualité des analyses de biologie médicale. Il a été estimé que sur les 257 laboratoires ayant participé à l'étude, seulement 83,7% étaient capables de diagnostiquer la loase sur la base de l'examen d'un frottis sanguin coloré au MGG (ANSM 2015). De plus, le

diagnostic de la loase doit être quantitatif. En effet, le principal problème de la loase aujourd'hui est qu'une microfilarémie élevée (supérieure à 30 000 mf/mL ou 1 000 mf/50µL) expose à des effets indésirables potentiellement mortels lors de la prise d'ivermectine (Gardon, Gardon-Wendel, Demanga-Ngangue, et al. 1997; Chesnais et al. 2020) ; la quantification doit donc être précise. En France, pour qu'un laboratoire de biologie médicale puisse demander l'accréditation d'une de ses techniques quantitatives, il doit répondre à plusieurs exigences (si applicables) telles que : l'exactitude (étroitesse de l'accord entre la moyenne d'un nombre infini de valeurs mesurées répétées et une valeur de référence), la fidélité (répétabilité et reproductibilité de la méthode), la robustesse (mesure de la capacité d'une procédure analytique à ne pas être affectée par des variations faibles mais délibérées des paramètres de la méthode) et la corrélation avec une méthode déjà utilisée dans le laboratoire (différence globale d'exactitude) (Roques et al. 2010). Au cours d'une étude de terrain à Sibiti, en République du Congo, nous avons effectué un dépistage de la microfilarémie à *L. loa* au sein de la population. A partir de ces dépistages, nous avons évalué une des exigences nécessaires à l'accréditation : la fidélité (répétabilité et reproductibilité) de cette méthode de diagnostic auprès de deux techniciens selon les critères recommandés par le comité français d'accréditation des laboratoires d'analyses médicales (COFRAC).

### 6.5.2 Méthode

#### *6.5.2.1 Personnel et site d'étude*

Cette étude a été menée dans le secteur opérationnel de la santé de Sibiti, en République du Congo. Nous avons invité deux lecteurs confirmés à participer au projet. Cette étude a été réalisée de janvier à avril 2021 à partir de la banque d'échantillons des personnes dépistées à Sibiti dans le cadre d'un essai clinique évaluant le lévamisole sur la loase.

#### *6.5.2.2 Lames positives à Loa loa*

Lors de l'essai clinique, 1876 lames présentant des microfilaires à *Loa loa* ont été collectées. Pour chaque point de prélèvement et chaque patient, le technicien a réalisé deux lames successives à partir de deux tubes à hématocrite remplis sur le même point de piqûre au

bout du doigt. Toutes les lames ont été lues en aveugle par deux techniciens expérimentés (4 lectures sur 2 lames différentes pour chaque point de prélèvement par patient).

#### *6.5.2.3 Répétabilité de la méthode*

Le test de répétabilité consiste à réaliser l'analyse du même échantillon pour la même analyse dans des conditions standardisées (même technicien, mêmes conditions matérielles et même jour). Le test porte sur l'analyse de 10 lames lues 3 fois dans la même journée, par le même technicien et avec le même microscope.

Une personne indépendante a sélectionné aléatoirement 10 lames parmi nos lames positives (4 avec une microfilarémie inférieure à 999 microfilaires par mL, 3 avec une microfilarémie comprise entre 1000 et 14999 mf/mL et 4 avec une microfilarémie comprise entre 15000 et 70000 mf/mL) puis les a anonymisées. Après lecture en aveugle par le technicien, les 10 lames ont été retournées à la personne indépendante qui les a mélangées et renommées. Ce processus a été appliqué trois fois au total (pour chaque lecteur). Ces lames ont été anonymisées et lues successivement par deux techniciens qualifiés, l'un ayant l'habitude de lire des lames de *L. loa* et l'autre, non. Ces lectures nous ont permis de déterminer la variabilité de lecture entre les techniciens. Ces tests de répétabilité nous ont permis de définir la fiabilité de la méthode en définissant un coefficient de variation (CV) appelé Coefficient de Répétabilité obtenu en divisant l'écart-type des mesures produites par leur moyenne.

#### *6.5.2.4 Reproductibilité de la méthode*

Le test de reproductibilité consiste à réaliser l'analyse du même échantillon pour la même analyse dans des conditions différentes. Il s'agit de trois conditions changeantes : technicien différent, jour de lecture différent, microscope différent. Pour chacun de ces 3 tests, l'analyse a été faite sur 10 lames choisies au hasard. Deux techniciens ont lu le même jour, sur le même microscope, les 10 mêmes lames. Un technicien a lu, deux jours différents, 10 lames avec le même microscope. Un technicien a lu le même jour 10 lames avec deux microscopes différents.

Ces tests de reproductibilité nous ont permis de définir la fiabilité de la méthode en définissant un CV appelé Coefficient de Fidélité Intermédiaire obtenu en divisant l'écart-type des mesures produites par leur moyenne.

#### *6.5.2.5 Valeurs de coefficient de variation acceptables*

A notre connaissance, il n'existe pas de limites d'acceptation consensuelles adaptées à la quantification de la microfilarémie à *L. loa* concernant la répétabilité et la fidélité intermédiaire. Selon les recommandations de la Société Française de Biochimie Clinique (SFBC), la limite d'acceptabilité de la fidélité intermédiaire doit être 1,33 fois supérieure à celle de la répétabilité (Ricos et al. 2014).

S'il n'existe pas de limites acceptables consensuelles pour un paramètre étudié, certains auteurs préconisent d'utiliser les formules suivantes : $f(x) = \frac{1}{\sqrt{x}}$ et $f(x) = \frac{1{,}33}{\sqrt{x}}$, respectivement pour la répétabilité et la fidélité intermédiaire où x représente la valeur moyenne d'éléments (microfilaires) retrouvée lors des essais afin de prendre en compte les variations de CV en fonction du nombre d'éléments à compter (Tachoires 2015). Pour nos analyses, nous avons raisonné à partir des valeurs moyennes du nombre de microfilaires retrouvées par lame et à partir des strates de microfilarémie dont étaient issus les lames retrouvées sur la lame afin de définir un CV acceptable global.

#### *6.5.2.6 Réutilisation des lames de l'essai clinique*

Afin de consolider nos résultats, nous avons évalué la variabilité dans les résultats microscopiques de toutes les lames réalisées lors de l'essai et lues en double lecture par 2 techniciens expérimentés différents.

### 6.5.3 Résultats

Les techniciens expérimenté et non expérimenté ont eu des coefficients de répétabilité de 15,3% et 13,0%, respectivement (tableau 57). En ce qui concerne les tests de reproductibilité, lorsque le jour de lecture ou le microscope était changé, les coefficients de fidélité intermédiaire étaient respectivement de 10,7% et 10,3%. Lorsque le technicien a été changé, nous avons obtenu le plus haut CV : 19,5% (tableau 58).

La figure 34 représente les coefficients de répétabilité retrouvés et les coefficients jugés acceptables par rapport aux nombres d'éléments moyens à compter par lame par application de la formule définie dans la partie Méthodes.

La figure 35 représente les coefficients de fidélité intermédiaire retrouvés et des coefficients jugés acceptables par rapport aux nombres d'éléments moyens à compter par lame par application de la formule définie dans la partie Méthodes.

Lors de cette étude, nous avons sélectionné en amont 4 lames correspondant à une microfilarémie comprise entre 1 et 50 mf/50µL, 3 lames correspondant à une microfilarémie comprise entre 51 et 750 mf/50µL et 4 lames correspondant à une microfilarémie comprise entre 751 et 3500 mf/50µL. Par application de la formule définie dans la partie Méthodes à partir des valeurs moyennes de ces intervalles, le coefficient de répétabilité acceptable est estimé à 9,4% et le coefficient de fidélité intermédiaire à 12,5% (9,4% x 1,33)[1].

L'analyse de la variabilité inter-lecture des 1876 lames positives lues en double lecture par deux techniciens différents donnent un coefficient de fidélité intermédiaire moyen de 13,2%. Le coefficient de fidélité intermédiaire moyen acceptable est estimé à 18,6%. La figure 36 reprend les coefficients de fidélité intermédiaire observés et les coefficients de fidélité intermédiaire acceptables estimés à partir de la formule définie en méthodes. Parmi les 1876 coefficients de fidélité intermédiaire estimés, 1219 (67,1%) sont inférieurs aux coefficients de fidélité acceptables estimés. Le tableau 59 présente les CV observés et acceptables et les pourcentages de double-lecture ayant un coefficient de fidélité estimé inférieur à celui acceptable en fonction des interquartiles des microfilarémies moyennes lues.

[1] $Coefficient\ de\ répétabilité\ acceptable = \frac{\left[4\times\left(1/\sqrt{\frac{1+50}{2}}\right)\right]+\left[3\times\left(1/\sqrt{\frac{51+750}{2}}\right)\right]+\left[4\times\left(1/\sqrt{\frac{751+3500}{2}}\right)\right]}{10}\times 100$

| **Technicien expérimenté** | | | | **Technicien non expérimenté** | | | |
|---|---|---|---|---|---|---|---|
| Lame | **Première lecture** (mf/50µL) | **Deuxième lecture** (mf/50µL) | **Troisième lecture** (mf/50µL) | Lame | **Première lecture** (mf/50µL) | **Deuxième lecture** (mf/50µL) | **Troisième lecture** (mf/50µL) |
| 1 | 5 | 7 | 5 | 1 | 6 | 6 | 3 |
| 2 | 10 | 10 | 4 | 2 | 7 | 7 | 5 |
| 3 | 22 | 20 | 17 | 3 | 19 | 22 | 14 |
| 4 | 17 | 21 | 24 | 4 | 27 | 24 | 19 |
| 5 | 70 | 70 | 52 | 5 | 70 | 63 | 58 |
| 6 | 124 | 123 | 93 | 6 | 127 | 124 | 111 |
| 7 | 389 | 371 | 355 | 7 | 373 | 355 | 341 |
| 8 | 1039 | 1065 | 801 | 8 | 934 | 919 | 890 |
| 9 | 1399 | 1380 | 1263 | 9 | 1449 | 1381 | 1400 |
| 10 | 3080 | 3212 | 3208 | 10 | 3275 | 3231 | 3289 |
| **Coefficient de répétabilité** = 15,3% | | | | **Coefficient de répétabilité** = 13,0% | | | |

**Tableau 57. Résultats de l'essai de répétabilité**

| **Jour différent** | | **Technicien différent** | | **Microscope différent** | |
|---|---|---|---|---|---|
| **Premier jour** (mf/50µL) | **Deuxième jour** (mf/50µL) | **Premier technicien** (mf/50µL) | **Deuxième technicien** (mf/50µL) | **Premier microscope** (mf/50µL) | **Second microscope** (mf/50µL) |
| 6 | 4 | 5 | 7 | 91 | 104 |
| 1 | 1 | 27 | 10 | 34 | 38 |
| 32 | 18 | 99 | 62 | 36 | 45 |
| 37 | 30 | 66 | 74 | 1608 | 1970 |
| 60 | 61 | 175 | 200 | 2012 | 2242 |
| 102 | 96 | 419 | 541 | 640 | 726 |
| 2095 | 2002 | 338 | 402 | 493 | 588 |
| 648 | 632 | 375 | 419 | Mal coloré | Mal coloré |
| 1050 | 1012 | 662 | 634 | 61 | 66 |
| 209 | 178 | 742 | 600 | 207 | 241 |
| **Coefficient de fidélité intermédiaire** = 10,7% | | **Coefficient de fidélité intermédiaire** = 19,5% | | **Coefficient de fidélité intermédiaire** = 10,3% | |

**Tableau 58. Résultats de l'essai de reproductibilité**

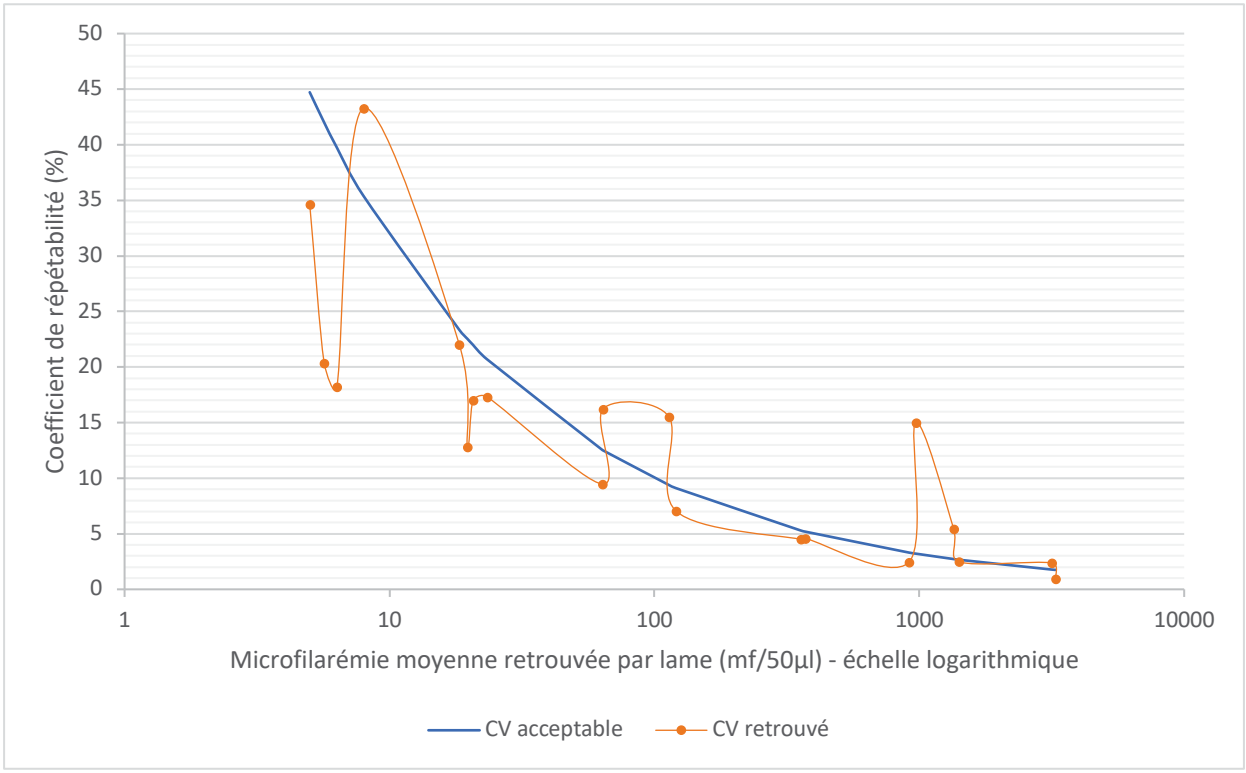


**Figure 34. Comparaison des coefficients de répétabilité observés aux coefficients acceptables calculés**

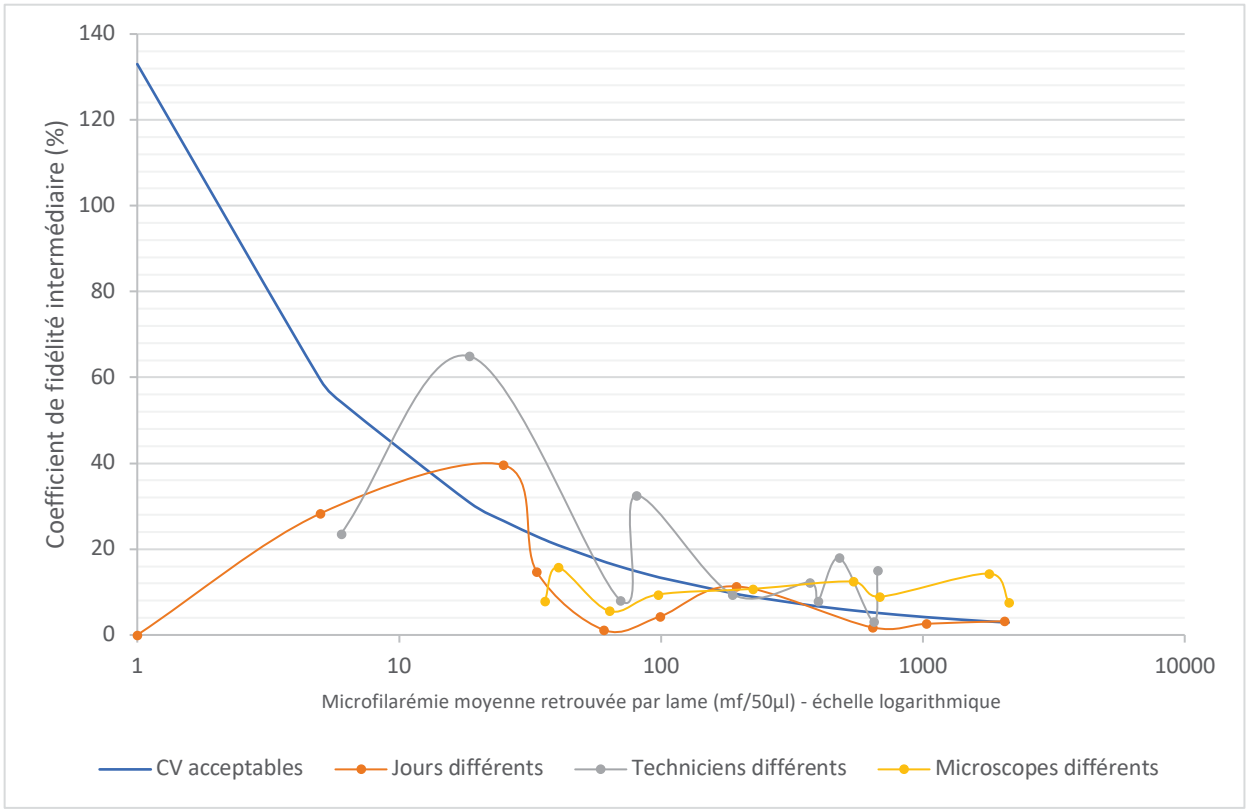


**Figure 35. Comparaison des coefficients de fidélité intermédiaire observés aux coefficients acceptables calculés**

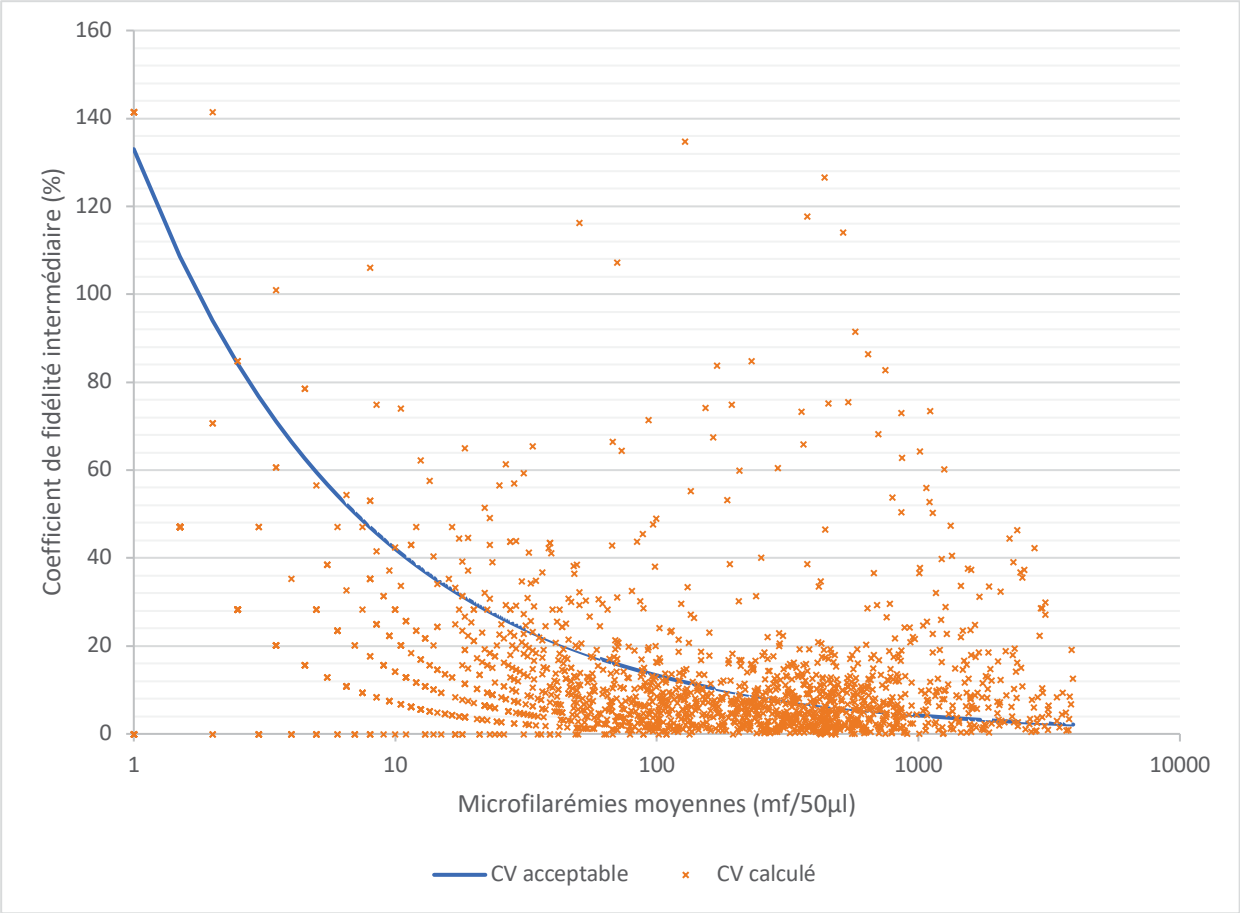


**Figure 36. Coefficients de fidélité intermédiaire observés selon les microfilarémies moyennes des lames positives et estimation des coefficients de fidélité intermédiaire acceptables**

| | Microfilarémies (mf/50µL) | | | |
|---|---|---|---|---|
| | **1 – 34,5** | **35 – 139,5** | **140 – 454** | **> 454** |
| CV acceptable moyen (%) | 45,9% | 16,0% | 8,2% | 4,4% |
| CV estimé (%) | 20,9% | 11,2% | 9,2% | 11,5% |
| CV estimé < CV acceptable (N, %) | 392 (86,3%) | 365 (80,4%) | 306 (67,4%) | 156 (34,4%) |

**Tableau 59. Coefficients de fidélité intermédiaire en fonction des interquartiles des moyennes de microfilarémie lues**

### 6.5.4 Discussion

A notre connaissance, nous avons réalisé la première étude portant sur la définition de certains critères nécessaires à l'accréditation de la méthode de diagnostic de la loase par microscopie. Nos résultats nous permettent d'estimer pour la première fois, les coefficients de répétabilité et fidélité intermédiaire de cette méthode. Nous estimons le coefficient moyen de répétabilité à 14,2% et le coefficient moyen de reproductibilité à 13,5%.

Comme discuté dans la partie Méthodes, il n'existe pas de référentiels permettant d'évaluer si nos CV sont acceptables ou non. A partir de méthodes utilisées par certains laboratoires, nous avons estimé les CV moyens acceptables dans notre étude, ils sont de 9,2 et 12,5%, respectivement pour la répétabilité et la reproductibilité. Cependant, ces approximations sont à réétudier car aucun consensus n'existe sur le sujet. En 2014, Ricos *et al*. ont réalisé une méta-analyse à partir de la littérature scientifique reprenant les valeurs des différents paramètres de qualité à évaluer plus de 300 analytes biologiques (Ricos et al. 2014). La microfilarémie à *L. loa* n'en faisait pas partie. Si on s'intéresse aux méthodes de comptage par microscopie, les valeurs de coefficient de répétabilité acceptables sont de 28,0%, 21,0%, 10,2%, 17,8%, 9,1% et 17,1% pour les polynucléaires basophiles, les polynucléaires éosinophiles, les lymphocytes, les monocytes, les plaquettes et les polynucléaires neutrophiles, respectivement. Ce qui laisse penser que nos CV moyens acceptables sont bas.

Le COFRAC indique que des résultats proches entre répétabilité et reproductibilité sont une manifestation de la robustesse de la méthode (COFRAC 2015). En effet, la répétabilité caractérise la meilleure performance possible (conditions optimales), alors que la reproductibilité est une mesure lors de conditions variables (opérateurs, matériel, ...). Dans notre étude, le coefficient moyen de répétabilité et le coefficient moyen de reproductibilité sont proches, allant dans le sens d'une bonne robustesse de la technique (mesure de la capacité d'une procédure analytique à ne pas être affectée par des variations faibles mais délibérées des paramètres de la méthode).

Les coefficients de variation estimés à partir de l'ensemble des lames lues au cours de l'essai clinique donnent un coefficient de variation moyen estimé inférieur au coefficient de variation acceptable calculé (13,2% et 18,6%, respectivement). Lorsqu'on s'intéresse aux résultats par classe de microfilarémies, on s'aperçoit que les lames aux faibles densités

parasitaires sont moins sujettes aux variations de lecture que les lames aux fortes densités parasitaires.

Cette étude conduit à deux principaux constats : (i) un besoin de formation en Afrique centrale, zone endémique de la loase, afin de pouvoir réduire cette variabilité par l'explication des bonnes pratiques de lecture microscopique et (ii) un besoin de trouver une méthode de lecture alternative plus calibrée, plus rapide et plus simple, permettant de réduire les variations inter-lecteurs et intra-lecteurs, notamment pour les microfilarémies hautes.

## 6.6 Conclusion

Le chapitre 6 de ce manuscrit de thèse s'est intéressé à la périodicité, aux variabilités à court et long terme de la microfilarémie à *L. loa* ainsi qu'à la variabilité dans les lectures de gouttes épaisses calibrées.

Bien comprendre la variabilité et la périodicité de la microfilarémie à *Loa loa* est primordial pour évaluer l'action d'interventions sur celle-ci. Comme mentionné précédemment, très peu d'études se sont intéressées au sujet, et la plupart d'entre elles évaluaient la microfilarémie au cours du temps d'un point de vue communautaire. Nos différentes études ancillaires à l'essai clinique ont permis de conforter la conclusion de ces études antérieures : la microfilarémie à *L. loa* reste stable dans le temps, d'un point de vue collectif.

Lorsqu'on s'intéresse à la périodicité de la microfilarémie à *L. loa* au niveau collectif, nous retrouvons classiquement une augmentation progressive dès le matin jusqu'à un pic aux alentours de midi et une diminution progressive jusqu'au soir. En théorie, il serait donc possible de prédire la microfilarémie d'un individu à n'importe quelle heure à partir d'un prélèvement à un instant *t.* En regardant les évolutions individuelles des microfilarémies de nos 13 patients au cours de la journée, nous pouvons constater une disparité importante. Plusieurs hypothèses peuvent être proposées: l'existence de mécanismes immunitaires essayant de réguler l'infection, l'existence de plusieurs foyers d'infection au sein d'une même communauté ; l'existence de plusieurs espèces d'insectes vecteurs aux heures de piqûre différentes ; l'influence des habitudes de vie de la personne (heure du lever, du coucher, repas…). Nous faisons également l'hypothèse que la température corporelle joue une part importante dans l'élévation de la microfilarémie au cours de la journée ; température qui peut varier au cours de la vie d'un individu (cycles menstruels, maladies infectieuses, ménopause…) pouvant ainsi provoquer des variations au long cours de la microfilarémie individuelle.

Nos études ont également permis de mettre en évidence l'existence, au sein des communautés, d'individus ayant une microfilarémie variable dans le temps. Une des problématiques importantes sur le sujet est qu'il n'existe pas, à ce jour, de définition de « variabilité dans le temps » concernant la microfilarémie à *L. loa*. Nos différentes analyses n'ont pas mis en évidence de facteurs extérieurs évidents (température, sexe, âge, heure de prélèvement, température extérieure) pouvant expliquer cette variabilité à court terme ou à long

terme. A long terme (année), les microfilarémies des hommes ont augmentées lorsqu'on raisonne en différence relative mais cette différence disparait en différence absolue, posant le problème d'utiliser les différences relatives sur des petites microfilarémies.

Le principal facteur qui semble avoir un impact sur la variabilité au cours du temps est la microfilarémie initiale. A long terme, les individus ayant plus de 12 000 mf/mL ont tendance à voir leur microfilarémie diminuer au cours du temps. A très court terme (5 jours), les individus ayant entre 5000 et 11 999 mf/mL ont tendance à voir leur microfilarémie diminuer. Cependant, cette tendance n'est pas confirmée à plus long terme.

Les hypothèses pouvant expliquer ce phénomène de variabilité chez certains individus sont encore à explorer. Le ver femelle de *L. loa* est capable de libérer jusqu'à 10 000 microfilaires par jour et pourrait donc potentiellement libérer des microfilaires de façon périodique dans le temps, juste avant un point de mesure et contribuer à cette variabilité.

Il est décrit que l'hôte peut, par l'intermédiaire de son système immunitaire, réguler le nombre de larves infectieuses de *L. loa* lors d'inoculations répétées (Wenk 1991), ce qui permet, *in fine*, d'obtenir un équilibre dans la charge parasitaire. Si le système immunitaire d'un individu est altéré, ce phénomène de régulation pourrait dysfonctionner et engendrer une augmentation progressive de la charge microfilarienne. Les charges communautaires en *L. loa* sont indéniablement stable dans le temps au sein d'une même communauté. Les individus ayant varié au cours du temps peuvent avoir changé leur habitude ou lieu de vie, augmentant ou diminuant leur exposition aux insectes vecteurs. Enfin, il existe un phénomène de variation aléatoire pouvant être due à la nature du flux sanguin, à la variabilité biologique physiologique, à la qualité du prélèvement ou de l'étalement ou encore à d'autres facteurs non contrôlables.

La variabilité étudiée concernait aussi la variabilité de résultats rendus à partir des gouttes épaisses calibrées. A notre connaissance, il s'agit de la première étude s'étant intéressé à l'exploration d'éléments nécessaires à l'accréditation d'une technique de biologie médicale pour le diagnostic microscopique de la microfilarémie à *L. loa*. Aucun consensus n'a fixé de valeur limites acceptables pour la variation en termes de répétabilité et de reproductibilité des résultats. Nous avons défini nous-même nos limites d'acceptabilité à partir des recommandations de la SFBC. Cependant, la microfilarémie à *L. loa* possède la particularité de pouvoir varier entre de très petites densités et de très fortes densités ; phénomène assez rare en pratique en biologie médicale. Il nous semble important de communiquer sur l'importance de

trouver un consensus permettant de fixer des valeurs limites adaptées afin de pouvoir s'assurer de la qualité du rendu des résultats lors de campagnes de dépistage pour la loase. Nous mettons en évidence une variabilité intra- et inter-lecteur au-dessus des limites acceptées calculées lors des essais de reproductibilité et de répétabilité. Lorsqu'on regarde les résultats issus de l'ensemble des lames positives lues lors de l'essai clinique, nous mettons en évidence un coefficient de variation acceptable, en moyenne globale et lors des lectures petites densités. Lors des lectures des plus grandes densités, nous mettons en évidence une variabilité plus importante. Lire une lame avec une charge parasitaire élevée est un travail laborieux, on estime à environ 20 minutes le temps de lecture d'une seule lame pour un patient avec un nombre de microfilaires modéré à important, ce qui peut logiquement, engendrer des variations de résultats en fonction du nombre de lames consécutivement lues par le technicien. Cette variabilité pourrait potentiellement être minimisée par la mise en place d'une technique alternative de lecture par diminution du nombre de champs lus à partir de la création d'un algorithme.

# 7. Perspectives de recherche et conclusion

Les principaux objectifs de l'OMS concernant l'onchocercose et la filariose lymphatique à l'horizon 2030 sont :

- La certification de l'interruption de la transmission de l'onchocercose dans 12 pays, contre 4 en 2020. Pour atteindre cet objectif il est nécessaire de traiter les zones hypoendémiques pour l'onchocercose où la loase est coendémique, et qui étaient auparavant exclues des campagnes de traitement de masse.
- L'élimination de la filariose lymphatique en tant que problème de santé publique dans au moins 59 pays (80% des pays affectés).

La mise en place de stratégies alternatives afin de traiter de manière efficace et sûre les patients vivants en zone endémique à la loase et d'optimiser les programmes actuels afin d'accélérer la lutte pourrait permettre d'atteindre cet objectif.

Nous avons apporté des résultats qui pourront servir dans la lutte contre les filarioses au niveau scientifique mais également au niveau programmatique.

## 7.1 L'albendazole

Dans le contexte des traitements communautaires semestriels par albendazole contre la filariose lymphatique dans les zones où la loase en endémique, nous avons mis en évidence l'existence d'une relation dose-réponse évidente au niveau individuel. D'un point de vue programmatique, nos résultats promeuvent l'intérêt de maintenir une observance en population la plus importante possible afin d'accélérer l'élimination de la filariose lymphatique aux niveaux individuel et communautaire. Les programmes de lutte pourront utiliser nos résultats afin de mettre en place de campagnes de mobilisation sociale illustrant l'importance d'atteindre et de maintenir des taux élevés d'observance thérapeutique dans les programmes d'élimination de la filariose lymphatique.

Du point de vue scientifique, nos résultats apportent également, pour la première fois :

- Des données individuelles sur l'efficacité de l'administration semestrielle d'albendazole
- Des données permettant de comparer l'administration d'albendazole semi-annuelle à l'absence de traitement ou d'un traitement annuel sur la filariose lymphatique, données manquantes jusque-là.

Nous démontrons l'efficacité supérieure sur l'élimination durable de la filariose lymphatique et des géohelminthes de l'administration semi-annuelle d'albendazole par rapport à l'administration annuelle ou l'absence de traitement.

Enfin, nous apportons également des éléments temporels nouveaux (time-ratios) pouvant permettre de modéliser le temps nécessaire à une élimination de la filariose lymphatique (et des géohelminthes) dans une population à partir de facteurs connus (effectifs, observance, niveau d'infection initial, …). Ces nouveaux éléments pourraient avoir un rôle dans l'évaluation des temps nécessaires avant de mettre en place les campagnes d'évaluation de la transmission.

## 7.2 Le lévamisole

Notre étude de pharmacovigilance sur le lévamisole met en évidence une très faible proportion d'effets indésirables graves lors de son administration en dose unique ou en tant qu'antihelminthique.

Lors de l'essai clinique sur le lévamisole, nous n'avons pas mis en évidence d'effets indésirables graves au sein de la population d'étude.

Ces deux résultats permettent de mettre en évidence le bon profil de sécurité du lévamisole lorsqu'il est utilisé pour son action antihelminthique à une posologie faible et ouvrent des perspectives d'utilisation pour cette molécule, comme, notamment son utilisation en tant qu'alternative aux benzimidazolés dans certaines zones où l'émergence de la résistance à ces molécules est redoutée en raison de la forte pression médicamenteuse causée par leur administration massive.

Une revue exhaustive des différentes études évaluant l'efficacité du lévamisole sur l'onchocercose et la filariose lymphatique a été réalisée. Elle met en évidence un effet faible à modéré de doses de lévamisole comprises entre 0,5 et 2,5 mg/kg en administration unique ou répétée sur la microfilarodermie à *O. volvulus*. Elle met également en évidence une efficacité importante du lévamisole sur *W. bancrofti* et *B. malayi*. Sur la filariose lymphatique, les doses utilisées oscillaient entre 0.5 et 6 mg/kg et les schémas d'administrations étaient soit une administration unique soit des administrations répétées. Dans tous les cas, le nadir de la microfilarémie semblait survenir entre le lendemain et le 3$^{ème}$ jour de la fin de l'administration.

Nous avons évalué, pour la première fois, l'innocuité et l'efficacité d'une dose unique de lévamisole (1,0, 1,5 et 2,5 mg/kg) sur la microfilarémie à *L. loa*. Nos résultats sont prometteurs : aucun effet secondaire grave n'est survenu chez des individus ayant des microfilarémies de 1 à 80 000 mf/mL ; nous mettons en évidence une diminution de la microfilarémie à *L. loa* faible à modérée lors de la semaine suivant la prise d'une dose unique de lévamisole.

L'efficacité du lévamisole observée lors de l'essai est trop faible pour imaginer l'utiliser comme traitement pour la loase. D'autres études sont nécessaires afin d'évaluer d'autres schémas d'administration.

En effet, une des importantes perspectives de recherche émanant de ces travaux de thèse est l'utilisation d'autres schémas d'administration du lévamisole afin de diminuer, de manière sûre, la microfilarémie à *L. loa* en dessous du seuil permettant par la suite un traitement par ivermectine. Nous voulons mettre en place une étude comparant l'efficacité et la tolérance du lévamisole à 2,5 mg/kg et 3,0 mg/kg pendant 3 et 5 jours sur des individus ayant de hautes microfilarémies.

Si nos essais à venir sont concluants, le lévamisole pourrait avoir une place dans l'arsenal thérapeutique de la prise en charge de la loase, en individuel au niveau hospitalier et dans les programmes de lutte, laissant espérer l'hypothèse de l'émergence d'une nouvelle stratégie de traitement alternative dans les zones où l'onchocercose ou la filariose lymphatique sont coendémiques avec la loase.

## 7.3 L'ivermectine

L'ivermectine est incontestablement un médicament qui a révolutionné la lutte contre les filarioses. Cette molécule possède de nombreux effets thérapeutiques, pour la plupart bien décrits et quantifiés mais certains, notamment son potentiel effet prophylactique n'ont pas fait l'objet d'études approfondies. Nous avons apporté la preuve qu'un traitement à fréquence trimestrielle est plus efficace pour prévenir l'apparition de nodules onchocerquiens qu'un traitement annuel, laissant penser que l'ivermectine possède effectivement un effet prophylactique qui pourrait être utilisé lors la réalisation de campagnes d'administration plus fréquentes. De plus, l'utilisation de l'ivermectine comme chimioprophylaxie à destination des

voyageurs et des expatriés pourrait être envisageable mais devra être évaluée par le biais d'études complémentaires.

Par le biais d'une étude de pharmacovigilance, nous mettons en évidence la survenue d'encéphalopathies post-ivermectine hors zones d'endémie à *L. loa*. Néanmoins, la compréhension des mécanismes mis en jeu nécessite des études complémentaires, qui pourraient permettre de comprendre également certains facteurs de risque, physiologiques ou génétiques, augmentant le risque de survenue d'encéphalopathies chez des patients microfilarémiques pour *L. loa*.

## 7.4 Variabilité et périodicité de la microfilarémie à *L. loa*

L'amélioration de la caractérisation de la périodicité et de la variabilité dans le temps de la DMF à *L. loa* est un point primordial dans la lutte contre cette filariose, puisqu'elle permettrait de faciliter la mise en place d'études longitudinales évaluant l'impact d'intervention sur ce parasite. Nous avons réalisé la revue des informations sur ce sujet et avons apporté quelques nouveaux éléments. Comme les auteurs l'ayant antérieurement étudié, nous retrouvons une microfilarémie stable au niveau collectif avec, cependant, certains individus présentant une variation plus ou moins importante de leur microfilarémie au cours du temps, à court, moyen et long terme.

Par ailleurs, nous avons modélisé la périodicité de la microfilarémie à *L. loa* et avons étudié, pour la première fois, la relation entre température ambiante, température corporelle et microfilarémie. Nous faisons l'hypothèse qu'il existe une association entre la température corporelle d'un individu et la périodicité selon laquelle sa microfilarémie évolue.

Une perspective de recherche émanant de ces travaux serait alors d'utiliser des modèles alternatifs au modèle cosinor afin de s'affranchir de ses limites et, *in fine*, d'imaginer l'utilisation d'équations afin d'extrapoler la microfilarémie d'un individu à un instant *t+X* à partir de sa microfilarémie à un instant *t* et de caractéristiques spécifiques à l'individu. Enfin, une étude longitudinale évaluant la variabilité individuelle de la DMF à *Loa loa* avec des temps de prélèvement définis pourrait permettre de mieux comprendre cette variabilité et les facteurs pouvant l'influencer.

## 7.5 Diagnostic microscopique

Nous avons évalué, pour la première fois, la variabilité intra- et interindividuelle du diagnostic microscopique de la microfilarémie à *L. loa*. Ces premiers résultats pourront servir de base à l'élaboration d'un consortium autour des coefficients de variation acceptables. Il nous semble important de commencer à valider la technique de diagnostic quantitatif microscopique de la microfilarémie à *L. loa* en validant les points demandés par les instituts d'accréditation de biologie médicale et d'estimer la part de variabilité de cette technique afin de pouvoir, par la suite, évaluer des techniques alternatives de lecture et de les comparer à ce « gold standard » actuel. En effet, nous avons mis en évidence une variabilité modérée à importante lors des double-lectures de lames fortement chargées en microfilaires de *L. loa*. Nous avons comme perspective de recherche de définir une nouvelle technique de lecture, par réduction du nombre de champs lus à partir d'un algorithme décisionnel.

Cette étude consistera à diviser la surface totale de la goutte épaisse calibrée en plusieurs champs de taille identique et standardisée, puis de dénombrer les microfilaires présentes dans chaque champ. Nous évaluerons dans quelle mesure, les comptages réalisés dans un nombre de champs réduits permette de prédire la densité microfilarienne avec précision. Cela pourra permettre d'identifier une possible hétérogénéité entre les champs de lecture d'une même lame et d'aider à la création d'un algorithme de lecture par la suite.

Si la technique alternative de lecture semble pertinente, des essais de reproductibilité et de répétabilité seront effectués de manière similaire à ceux présentés dans ce manuscrit afin de les comparer avec la méthode de référence. Dans un second temps, en déterminant des valeurs « critiques » pertinentes dans la prise en charge clinique des patients (< 2000 mf/mL, 2000-8000 mf/mL, 8000-30 000 mf/mL et > 30 000 mf/mL par exemple), la sensibilité, spécificité, valeur prédictive positive et valeur prédictive négative de cette technique alternative seront calculées par rapport à la lecture de lames entières. Pour finir, une étude médico-économique portant sur l'analyse comparative de deux stratégies diagnostiques sur la base de leurs coûts (temps technicien et coût des lames) et de la fiabilité de leurs résultats pourra être réalisée.

Ceci aurait un double intérêt : (i) la diminution des coûts des programmes de dépistage de la loase en augmentant le nombre de lames lues par jour et (ii) l'augmentation de la fiabilité

des résultats en diminuant la variabilité intra- et interindividuelle par la mise en place d'une technique calibrée et reproductible.

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# 9. Annexes

*Clinical Infectious Diseases*

MAJOR ARTICLE

# Results From 2 Cohort Studies in Central Africa Show That Clearance of *Wuchereria bancrofti* Infection After Repeated Rounds of Mass Drug Administration With Albendazole Alone Is Closely Linked to Individual Adherence

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***Background.*** Two community trials conducted from 2012 to 2018 in the Republic of Congo and the Democratic Republic of the Congo demonstrated the efficacy of semiannual mass drug administration (MDA) with albendazole (ALB) alone on lymphatic filariasis (LF). However, a high interindividual heterogeneity in the clearance of infection was observed.

***Methods.*** We analyzed trial data to assess the effect of individual adherence to ALB MDA on clearance of circulating filarial antigenemia (CFA) and microfilaremia. Community residents were offered a single dose of ALB every 6 months and tested for LF with a rapid test for CFA at baseline and then annually. CFA test results were scored on a semiquantitative scale. At each round, microfilaremia was assessed in CFA-positive individuals. All CFA-positive individuals for whom at least 1 follow-up measure was available were included in the analyses. Parametric survival models were used to assess the influence of treatment adherence on LF infection indicators.

***Results.*** Of 2658 individuals enrolled in the trials, 394 and 129 were eligible for analysis of CFA and microfilaremia clearance, respectively. After adjusting for age, sex, and initial CFA score, the predicted mean time for clearing CFA was shorter in persons who had taken 2 doses of ALB per year (3.9 years) than in persons who had taken 1 or 0 dose (4.4 and 5.3 years; $P < .001$ for both). A similar pattern was observed for microfilaremia clearance.

***Conclusions.*** These results demonstrate a clear dose-response relationship for the effect of ALB on clearance of CFA and microfilaremia.

**Keywords.** albendazole; lymphatic filariasis; mass drug administration; treatment adherence; parametric survival analysis.

Lymphatic filariasis (LF) is a mosquito-borne parasitic infection caused mainly by *Wuchereria bancrofti*. The strategy for LF elimination is to interrupt the transmission cycle between humans and vectors. In African countries where onchocerciasis is endemic, programs provide annual mass drug administration (MDA) with ivermectin (IVM) plus albendazole (ALB). Bednets are also often provided to limit mosquito exposure. Treatment with IVM and ALB reduces the density of the larval stages of the parasite (microfilariae [Mf]) in the blood. However, MDA has to be repeated for many years because these drugs have a limited efficacy for killing adult worms [1]. In areas where LF is coendemic with loiasis, another filarial infection caused by *Loa loa*, this strategy is dangerous because IVM can induce serious adverse events (SAEs) in people with very high *L. loa* microfilarial densities (MFD) [2]. In these areas, alternative strategies have to be implemented. Previous clinical trials that compared the effects of various drugs on *W. bancrofti* MFD suggested that treatment with ALB alone might reduce MFD, albeit at a slower rate than after combined treatment with IVM and ALB [3–13]. ALB does not induce SAEs in patients with high *L. loa* MFD [14–16]. In 2012, the World Health Organization (WHO) proposed that MDA with ALB (preferably semiannual, and combined with integrated vector management) might be used to eliminate LF in areas where loiasis is coendemic [17]. The results of 2 community trials conducted in the Republic of Congo (Congo) and the Democratic Republic of the Congo (DRC) confirmed that this strategy was effective. At the first site, where baseline circulating filarial antigenemia (CFA) and Mf prevalences were moderate

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(17.3% and 5.3%, respectively) and treatment adherence was high (83%–90%), these indicators decreased to 4.7% and 0.3%, respectively, after 3 years of semiannual MDA with ALB alone [18]. In DRC, where baseline infection prevalences were higher and treatment adherence lower (56%–88%), CFA and Mf prevalences decreased from 31.6% to 8.5% and from 12.0% to 0.9%, respectively, after 4 years of semiannual MDA with ALB [19]. Although MDA with ALB alone was highly effective at the community level, considerable heterogeneity was observed in parasite clearance at the individual level; some individuals cleared their infections rapidly, while others remained infected after 8 rounds of MDA. In this study, we reanalyzed data collected from these 2 community trials to assess the effect of individual adherence to ALB treatment on the CFA and *W. bancrofti* microfilaremia clearance rates.

## METHODS

### Study Populations

The design of the 2 studies has been described elsewhere [18, 19]. In Congo, the study was conducted from 2012 to 2015 in Seke-Pembe, a village located in Mabombo Health District (Bouenza division). In DRC, the study site consisted of 2 contiguous villages (Mbunkimi and Misay) located in the Kwilu province, and the trial took place from 2014 to 2018. Study participants were tested for LF infection at baseline and then annually. Both studies were approved by ethics committees and administrative authorities in the respective countries. Adult participants signed an informed consent form. Participants aged <18 years were enrolled only after verbal assent and if 1 parent signed a consent form.

A total of 2658 individuals were examined for LF infection at least once during the 2 studies. The present analysis included all individuals who were CFA-positive at the time of their first test (which was not necessarily performed during the year when the trial started at the site) and who had at least 1 subsequent examination. Therefore, individuals who had progressed from CFA-negative to CFA-positive during the follow-up period and those who were CFA-negative at all time points tested were not included in the analysis.

### Assessment of *W. bancrofti* Infection

Annual parasitological assessments were performed for participants aged ≥5 years. LF infections were detected by CFA testing using point-of-care tests. In Congo, testing was done with the BinaxNOW Filariasis immunochromatographic card test (ICT; Alere, Scarborough, ME) in 2012, 2013, and 2014 and with the Filariasis Test Strip (FTS; Alere, Scarborough, ME) in 2015. All antigen testing in DRC was performed with FTS. ICT and FTS results were scored semiquantitatively (0, 1, 2, or 3 according to the relative intensities of the test and control lines) [18, 20]. All CFA-positive individuals were invited to return for blood sampling between 10:00 PM and 1:00 AM for assessment of *W. bancrofti* microfilaremia. MFDs were based on the arithmetic mean of the counts of two 70-µL-thick blood smears and expressed as microfilariae per milliliter.

### Drug Distribution and Assessment of Treatment Adherence

CFA-negative individuals were treated with a single tablet of ALB (400 mg) immediately after antigen testing under the direct observation of investigators. Those with positive CFA test results were treated with ALB just after collection of night blood for Mf testing. Residents who had not participated in the parasitological survey were also offered ALB treatment.

All treatments were provided under the supervision of a local healthcare worker who was also responsible for conducting a population census before each semiannual MDA. Every treatment was recorded in a drug treatment register. In addition, during the annual assessment visits, we asked the participants if they had received ALB during the previous MDA campaign, which was 6 months earlier. Therefore, for each year of the study, we could determine for each participant whether he/she had taken 2, 1, or 0 ALB tablets.

### Sociodemographics and Risk Factors for LF

At inclusion, we collected information about sex and age. At each visit using a standardized 1-page questionnaire, we also collected sociodemographic characteristics and habits that are known to be risk factors for LF, such as bednet usage and occupation (fishing, hunting, and farming), and regularly sleeping outside of the village in the bush [21, 22].

### Statistical Analyses

The events analyzed are clearance of CFA (the transition from a positive to a negative CFA test during follow-up) and clearance of microfilaremia. We used survival analysis methods [23] to account for the individual follow-up nature of the data. The start date for the survival analysis was the first visit (index date). Individual observations were censored at the end of the follow-up or at the date of the event (date of the annual parasitological survey). Each participant's data were considered for calculation of cumulative person-years in the survival analysis.

We considered the following covariates for the analysis: sex, initial MFD (placed into 3 categories of similar sample size: 1 to 150, 150 to 300, and > 300 Mf/mL), initial CFA score (from 1 to 3), a history of fishing as an occupation (yes or no), and a history of regularly sleeping in the bush (yes or no).

We also considered the following time-varying covariates: age (categorized according to interquartile and median values: 5–17, 18–30, 31–45, and ≥46 years), number of ALB tablets taken during the previous year (0, 1, or 2), bednet use during the previous night (yes or no), and the CFA test used (ICT or FTS).

Univariate analysis of clearance rates was conducted using Mantel-Haenszel tests. Clearance rates represent the probability of occurrence of clearance in a specified period of time.

We used a parametric survival model with accelerated failure time [24] to estimate the influence of time-varying variables on

infection clearance (time-to-event) [25]. Several time distributions that do not require meeting the proportional risk assumption were tested according to the Akaike information criterion (AIC). For the survival models, random effects, at both village and household levels, were assessed using results of likelihood-ratio tests. Results are presented as time ratios with 95% confidence intervals. Time ratios represent time differences to event according to the reference category. Sociodemographic data, occupation, initial infection intensity (CFA and/or MFD), and the number of ALB tablets taken each year were included in the CFA and microfilaremia clearance survival models. The type of test (ICT or FTS) was included in the CFA clearance model. The fitted models used to estimate average times to clear CFA and microfilaremia included all explanatory variables.

A mixed model with random effect at the individual level was used to describe changes in MFD according to time, treatment history, and sociodemographic information. Several transformations (linear, quadratic, first-order fractional polynomials, and second-order fractional polynomials) were tested for the time variable, and selection was made according to the AIC. As for CFA clearance analysis, random effects at village and household levels were assessed. Last, the significance of relevant interaction terms was assessed (age and sex, age and initial CFA score, age and initial MFD, age and number of ALB treatments taken, sex and initial CFA score, sex and initial MFD, sex and number of ALB treatments taken) for CFA and microfilaremia clearance and MFD change analyses. All analyses were performed using STATA v.15.1 software (StataCorp, LP, College Station, TX).

## RESULTS

### Study Participants

Of the 2658 participants enrolled in the studies, 879 were tested only once; 22 who were CFA-negative at baseline acquired CFA (15 in DRC and 7 in Congo), and 1363 were CFA-negative at baseline and all follow-up times. Thus, observations from 394 participants were available for analysis of CFA clearance for a total of 1369 person-years of follow-up and 203 CFA clearance events. For the microfilaremia clearance analysis, 129 individuals had a total of 400 person-years of follow-up with 100 microfilaremia-clearance events. The survival data concerning nontime-varying variables are summarized in Table 1.

### CFA and Microfilaremia Clearance Rates

Clearance rates with significance values are presented in Table 2. The probability of CFA clearance was negatively correlated with initial CFA score, and the probability of microfilaremia clearance was negatively correlated with initial MFD. A history of sleeping regularly in the bush decreased the probability of CFA clearance. CFA clearance was

**Table 1. Survival Data for Time Constant Variables Used in Circulating Filarial Antigenemia and Microfilaremia Survival Models**

| | | CFA Clearance | | Microfilaremia Clearance | |
|---|---|---|---|---|---|
| Variable | Category | Person-years | No. of Events | Person-years | No. of Events |
| | | 1369 | 203 | 400 | 100 |
| Sex | Male | 754 | 112 | 232 | 61 |
| | Female | 615 | 91 | 168 | 39 |
| Age at inclusion, y | 5–17 | 304 | 47 | 87 | 22 |
| | 18–30 | 349 | 48 | 92 | 23 |
| | 31–45 | 402 | 59 | 113 | 24 |
| | ≥46 | 314 | 49 | 108 | 31 |
| CFA score at inclusion | 1 | 488 | 128 | 30 | 9 |
| | 2 | 352 | 51 | 91 | 28 |
| | 3 | 529 | 24 | 279 | 63 |
| Bednets use at inclusion | No | 561 | 82 | 138 | 33 |
| | Yes | 808 | 121 | 262 | 67 |
| Fishing activities at inclusion | No | 596 | 100 | 161 | 43 |
| | Yes | 699 | 95 | 211 | 52 |
| History of sleeping outside at inclusion | No | 953 | 159 | 248 | 63 |
| | Yes | 408 | 44 | 144 | 36 |
| Village | Misay (DRC) | 297 | 39 | 85 | 21 |
| | Mbunkimi (DRC) | 660 | 79 | 199 | 49 |
| | Seke Pembe (Congo) | 412 | 85 | 116 | 30 |
| Study site | Bouenza (Congo) | 957 | 118 | 284 | 70 |
| | Kwilu (DRC) | 412 | 85 | 116 | 30 |
| Initial microfilarial density | 0–150 Mf/mL | | | 144 | 44 |
| | 151–300 Mf/mL | | | 77 | 22 |
| | >300 Mf/mL | | | 179 | 34 |

Abbreviation: CFA, circulating filarial antigenemia; DRC, the Democratic Republic of the Congo; Mf, microfilariae.

**Table 2. Univariate Clearance Rates for Circulating Filarial Antigenemia and Microfilaremia**

| | | CFA | | | Microfilaremia | | |
|---|---|---|---|---|---|---|---|
| Variable | Category | Clearance Rate[a] | 95% CI | *P* Value[b] | Clearance Rate[a] | 95% CI | *P* Value[b] |
| | | 14.8 | 12.9 to 17.0 | | 25.0 | 20.5 to 30.4 | |
| Sex | Male | 14.8 | 12.0 to 18.2 | .989 | 26.3 | 20.4 to 33.8 | .543 |
| | Female | 14.8 | 12.3 to 17.9 | | 23.2 | 17.0 to 31.8 | |
| Age at inclusion, y | 5–17 | 15.5 | 11.6 to 20.6 | .859 | 25.3 | 16.6 to 38.4 | .739 |
| | 18–30 | 13.7 | 10.4 to 18.2 | | 25.0 | 16.6 to 37.6 | |
| | 31–45 | 14.7 | 11.4 to 18.9 | | 21.2 | 14.2 to 31.7 | |
| | ≥46 | 15.6 | 11.8 to 20.6 | | 28.7 | 20.2 to 40.8 | |
| CFA score at inclusion | 1 | 26.2 | 22.1 to 31.2 | <.0001 | 30.0 | 15.6 to 57.6 | .184 |
| | 2 | 14.5 | 11.0 to 19.1 | | 30.8 | 21.2 to 44.6 | |
| | 3 | 4.5 | 3.0 to 6.8 | | 22.6 | 17.6 to 28.9 | |
| Bednets use at inclusion | No | 14.6 | 11.8 to 18.1 | .677 | 23.9 | 17.0 to 33.6 | .752 |
| | Yes | 14.9 | 12.5 to 17.9 | | 25.6 | 20.1 to 32.5 | |
| Fishing activity at inclusion | No | 16.8 | 13.8 to 20.4 | .671 | 19.0 | 14.1 to 25.6 | .444 |
| | Yes | 13.6 | 11.1 to 16.6 | | 16.2 | 12.4 to 21.3 | |
| History of sleeping outside at inclusion | No | 16.7 | 14.3 to 19.5 | .042 | 26.7 | 19.8 to 36.0 | .696 |
| | Yes | 10.8 | 8.0 to 14.5 | | 24.6 | 18.8 to 32.2 | |
| Village | Misay (DRC) | 13.1 | 9.6 to 18.0 | <.0001 | 24.7 | 16.1 to 37.9 | .859 |
| | Mbunkimi (DRC) | 12.0 | 9.6 to 14.9 | | 24.6 | 18.6 to 32.6 | |
| | Seke Pembe (Congo) | 20.6 | 16.7 to 25.5 | | 25.9 | 18.1 to 37.0 | |
| Study site | Bouenza (Congo) | 12.3 | 10.3 to 14.8 | <.001 | 24.6 | 19.5 to 31.1 | .826 |
| | Kwilu (DRC) | 20.6 | 16.7 to 25.5 | | 25.9 | 18.1 to 37.0 | |
| Initial microfilarial density | 1–150 Mf/mL | | | | 30.6 | 22.7 to 41.0 | .036 |
| | 151–300 Mf/mL | | | | 28.6 | 18.8 to 43.4 | |
| | >300 Mf/mL | | | | 19.0 | 13.6 to 26.6 | |

Abbreviations: CFA, circulating filarial antigenemia; CI, confidence interval; DRC, the Democratic Republic of the Congo; Mf, microfilariae.

[a]Calculated for 100 person-years.

[b]*P* value is calculated from significance tests using the Mantel-Haenszel method based on stratified rate ratios.

also more likely in Congo than in DRC. The probabilities for clearance of CFA for each of the 39 treatment patterns during the 5-year period are included in the Supplementary Materials 1.

### Parametric Survival Multivariate Models for the Clearance of CFA and Microfilaremia

Results from the parametric survival model analyses are presented in Table 3. Log-logistic distribution and log-normal distribution were the best fits for time in the CFA and microfilaremia clearance models, respectively. No interactions between covariates were found. A random effect at the village level ($P = .0277$) was included in the CFA clearance model (intraclass correlation coefficient = 7.27%), but this was not significant in the microfilaremia clearance model ($P = .346$). CFA score at inclusion, frequently sleeping outdoors, and type of CFA test were all significantly associated with CFA clearance. Times to CFA clearance were significantly longer in individuals with higher initial CFA scores. Sleeping outdoors significantly increased the time to CFA clearance. Assessment of the CFA by ICT decreased the observed duration to CFA clearance. Predicted average time for clearing CFA was shorter in those who had taken 2 doses of ALB per year (3.9 years) than in those who had taken 1 or 0 dose (4.4 and 5.3 years, $P < .001$ for both comparisons). Microfilaremia clearance had a similar pattern: individuals who had taken 2 doses of ALB per year became amicrofilaremic after a mean time of 3.1 years, whereas those who had taken 1 or 0 dose per year needed 3.6 ($P < .001$) and 5.9 years ($P < .001$), respectively, to clear their microfilaremia. Time to microfilaremia clearance was also significantly longer in individuals with higher initial MFD.

### Changes in MFD Over Time

No transformation of time was required for the model. Neither the village- ($P = .496$) nor the household-level ($P = .529$) random effect was significant in the mixed model. Results from the mixed model with no random effect are presented in Table 4. MFD reduction was more rapid when individuals were adherent with MDA. The decrease in MFD was not significantly different in those who had taken 0 or 1 dose of ALB per year. Figure 1 shows predicted changes in MFD according to the number of doses taken per year with time transformation into a fractional polynomial of order 2 (see Supplementary Materials 2). All predictions were adjusted for sex, age, and initial MFD. Differences in slopes were highly significant between 0 and 2 doses of ALB ($P = .009$) and between 2 and 1 dose ($P = .004$) but not significant between 1 and 0 dose ($P = .419$).

**Table 3. Results From Parametric Survival Models for Circulating Filarial Antigenemia With Village as a Random Effect and Microfilaremia Clearance**

| | | CFA Clearance | | | Microfilaremia Clearance | | |
|---|---|---|---|---|---|---|---|
| Variable | Category | Adjusted Time Ratio | 95% CI | *P* Value | Adjusted Time Ratio | 95% CI | *P* Value |
| Sex | Female | Ref. | | | Ref. | | |
| | Male | 1.01 | .96 to 1.06 | .718 | 0.94 | .84 to 1.05 | .281 |
| Age, y | 5–17 | Ref. | | | Ref. | | |
| | 18–30 | 1.00 | .93 to 1.07 | .910 | 1.02 | .87 to 1.20 | .769 |
| | 31–45 | 1.02 | .95 to 1.08 | .515 | 1.15 | .98 to 1.34 | .082 |
| | ≥46 | 1.00 | .93 to 1.07 | .992 | 1.03 | .89 to 1.20 | .644 |
| Initial CFA score | 1 | Ref. | | | Ref. | | |
| | 2 | 1.14 | 1.08 to 1.20 | <.001 | 0.91 | .74 to 1.11 | .357 |
| | 3 | 1.40 | 1.31 to 1.51 | <.001 | 1.00 | .83 to 1.21 | .982 |
| Annual treatment | 0 dose | 1.35 | 1.26 to 1.45 | <.001 | 1.82 | 1.46 to 2.27 | <.001 |
| | 1 dose | 1.12 | 1.06 to 1.19 | <.001 | 1.18 | 1.04 to 1.34 | .008 |
| | 2 doses | Ref. | | | Ref. | | |
| Bednets | No | Ref. | | | Ref. | | |
| | Yes | 0.98 | .94 to 1.03 | .515 | 0.94 | .84 to 1.04 | .243 |
| Fishing | No | Ref. | | | Ref. | | |
| | Yes | 0.97 | .92 to 1.02 | .280 | 1.06 | .95 to 1.18 | .305 |
| Sleeping outside | No | Ref. | | | Ref. | | |
| | Yes | 1.09 | 1.03 to 1.16 | .002 | 1.05 | .94 to 1.18 | .397 |
| Test used | Filariasis Test Strip | Ref. | | | | | |
| | Immunochromatographic card test | 0.76 | .69 to .85 | <.001 | | | |
| Initial microfilarial density | 1–150 Mf/mL | | | | Ref. | | |
| | 151–300 Mf/mL | | | | 1.04 | .92 to 1.19 | .514 |
| | >300 Mf/mL | | | | 1.28 | 1.14 to 1.43 | <.001 |

Abbreviations: CFA, circulating filarial antigenemia; CI, confidence interval; Mf, microfilariae.

**Table 4. Mixed Model Results for the Evolution of Microfilariae Density**

| Variable | Category | Adjusted Coefficients | 95% Confidence Interval | *P* Value |
|---|---|---|---|---|
| Sex | Female | Ref. | | |
| | Male | −26.6 | −131.2 to 77.9 | .618 |
| Age, y | 5–17 | Ref. | | |
| | 18–30 | −93.1 | −236.6 to 50.4 | .203 |
| | 31–45 | 35.3 | −103.6 to 174.1 | .619 |
| | ≥46 | −59.6 | −196.8 to 77.5 | .394 |
| Initial circulating filarial antigenemia score | 1 | Ref. | | |
| | 2 | −37.8 | −250.9 to 175.4 | .728 |
| | 3 | 52.6 | −156.8 to 262.0 | .622 |
| Initial microfilarial density | 1–200 Mf/mL | Ref. | | |
| | > 200 Mf/mL | 221.3 | 124.3 to 318.3 | <.001 |
| Bednets | No | Ref. | | |
| | Yes | 29.9 | −61.9 to 121.8 | .523 |
| Fishing | No | Ref. | | |
| | Yes | −18.5 | −133.9 to 96.9 | .753 |
| Sleeping outside | No | Ref. | | |
| | Yes | −21.0 | −138.5 to 96.5 | .753 |
| Annual treatment | 0 dose | Ref. | | |
| | 1 dose | 177.8 | −472.3 to 828.0 | .592 |
| | 2 doses | 416.6 | −217.3 to 1050.5 | .198 |
| Time | Continuous | −22.0 | −174.6 to 130.5 | .777 |
| Annual treatment interacted with time | 0 dose | Ref. | | |
| | 1 dose | −68.6 | −234.9 to 97.7 | .419 |
| | 2 doses | −210.9 | −369.3 to −52.44 | .009 |

Abbreviation: Mf, microfilariae.

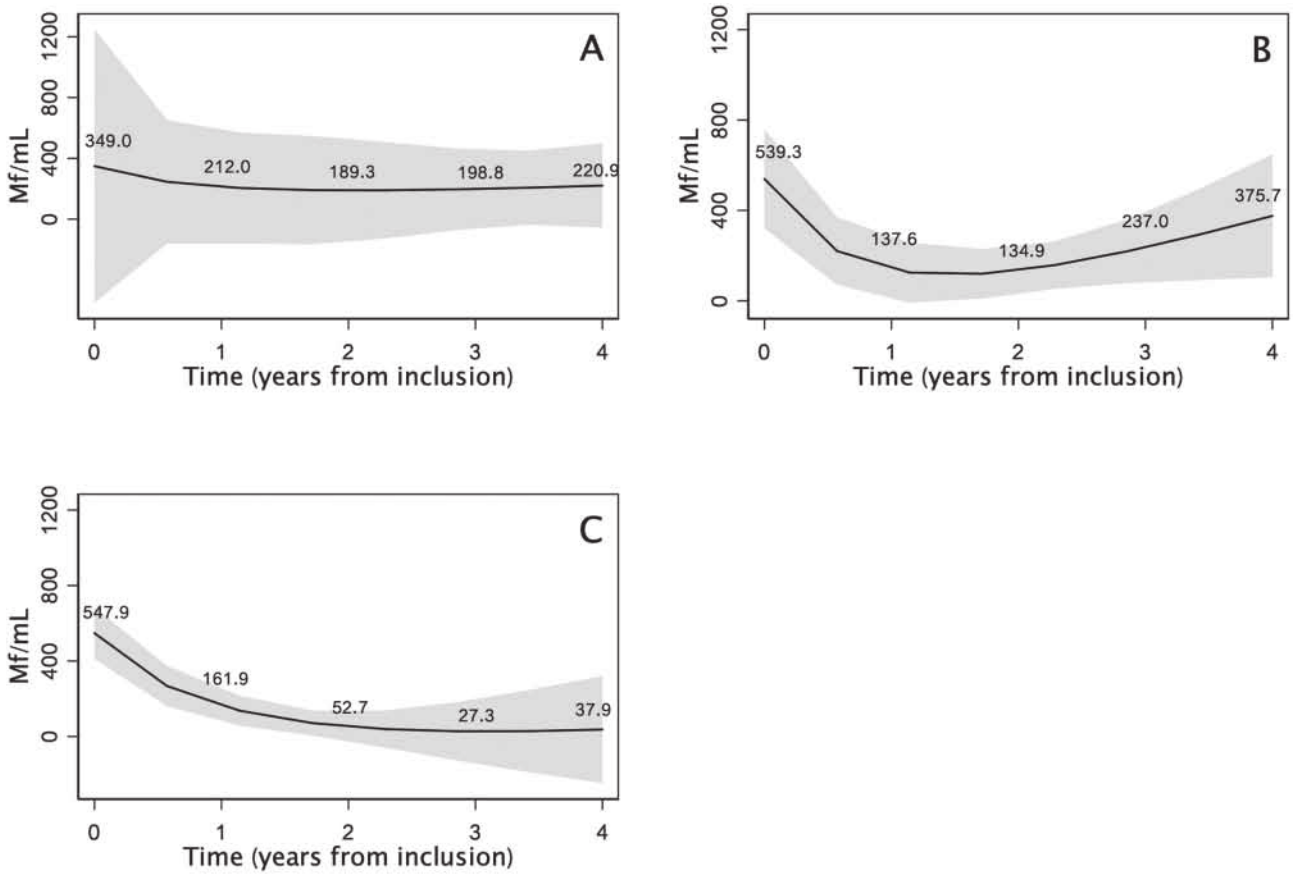


**Figure 1.** Predictions of Mf density evolution according to time (fractional polynomial of order 2) and adherence with mass drug administration (*A*, 0 dose per year; *B*, 1 dose per year; *C*, 2 doses per year). Full model and time transformations are available in Supplementary Materials 2. Abbreviation: Mf, microfilariae.

## DISCUSSION

The WHO's provisional recommendation to use semiannual MDA with ALB alone to control LF in areas where *L. loa* is coendemic was based on thin evidence. The few trials [5, 11–13] that had evaluated the effect of a single dose of ALB on LF infection had demonstrated a modest effect of the drug on MFD. In addition, 2 meta-analyses of the efficacy of a single dose of ALB alone on Mf and CFA prevalences concluded that this treatment would induce only a small to nonexistent decrease in these outcomes at 3, 6, or 12 months post-treatment [26, 27].

We conducted community trials in 2 settings to evaluate the impact of semiannual MDA with ALB on LF [18, 19]. However, a major methodological limitation of these trials was that the effect of semiannual treatment was not directly compared with that of annual treatment or no treatment. Thus, the analyses presented here provide important information regarding the added value of semiannual ALB treatment vs annual MDA or no treatment on LF infection parameters. Our longitudinal analyses from infected persons clearly demonstrate a dose-response effect for ALB treatment on CFA and on microfilaremia. Our results show that good adherence leads to faster clearance of LF infection in individuals. Both clearances were significantly associated with the number of doses of ALB taken annually and with initial infection levels. A lower initial CFA score was associated with a higher probability of CFA clearance. Therefore, the knowledge of the individual semiquantitative results at baseline may be useful to improve planning for LF elimination programs. The use of the ICT was associated with an increased probability of CFA clearance relative to use of the FTS, and this is likely due to the higher sensitivity of the FTS [28]. Although baseline CFA scores were not associated with more rapid clearance of microfilaremia, higher initial MFD increased the time required for total microfilaremia clearance.

Regarding an individual's exposure and habits, individuals who slept regularly outdoors took longer to clear CFA. This was probably due to reinfection; prior studies have identified sleeping outdoors as a significant risk factor for LF in central Africa [21, 22]. However, the use of bednets or a history of fishing were not significantly associated with the clearances. Although the nonuse of bednets has been shown to be a risk factor for LF infection, their use in infected individuals (without MDA) was not effective for clearing infections or for

reducing Mf prevalence in the time frames (3 or 4 years) of this study [29]. Data on the relationship between bednet usage and treatment adherence are included in Supplementary Materials 3. Hunting and agricultural activities were not included in the models because the numbers of hunters and farmers were small at the study sites and inclusion of these occupations would have destabilized the models. Individuals were more likely to clear CFA after MDA in Congo than in DRC. This might be due to the fact that therapeutic coverage was higher and more constant and baseline infection prevalence lower in Congo than in DRC, which may reduce transmission and, therefore, the probability that reinfection occurs.

The activity of ALB alone for LF has important implications for current protocols that rely heavily on CFA surveys in school-aged children for MDA-stopping decisions and post-MDA surveillance. Indeed, since soil-transmitted helminth programs routinely only treat children, using this demographic as a sentinel for MDA-stopping decisions may underestimate the level of community-wide transmission because LF will tend to be less prevalent in children who are treated more frequently than adults.

We elected to use parametric survival models to analyze these data because these models are more flexible and allow longitudinal analyses with time-varying variables (ie, ALB intake). In addition, they are more informative than nonparametric approaches because they provide time ratios, enable predictions of mean and median survival times, and have more power than semiparametric models. Log-logistic distribution for the CFA clearance model and log-normal distribution for the microfilaremia clearance model were the best fits for our data, and they have the advantage of not requiring proportional risk assumptions, unlike conventional Cox survival models.

The presence of bias cannot be excluded. Prevalence bias may be present. However, fewer than 11% of the population (8.5% and 10.4% for the CFA and microfilaremia clearance models, respectively) had taken ALB prior to our study. We believe that this bias, if it exists, would have had very little impact on our results. In addition, participation bias cannot be excluded: people with a high participation frequency in our study may have different characteristics than nonparticipants, including adherence with treatment.

We have mentioned that participation rates in MDA decreased over time, probably reflecting a type of fatigue on the part of some community members [19]. We believe that we have demonstrated through these new analyses that participation rates in MDA programs must be maintained at high levels to accelerate the elimination of LF in individuals and communities. Evidence from this study could be used in social mobilization programs to illustrate the importance of achieving and sustaining high rates of MDA adherence in LF elimination programs.

## Supplementary Data

Supplementary materials are available at *Clinical Infectious Diseases* online. Consisting of data provided by the authors to benefit the reader, the posted materials are not copyedited and are the sole responsibility of the authors, so questions or comments should be addressed to the corresponding author.

## Notes

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***Potential conflicts of interest.*** The authors: No reported conflicts of interest. All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest.

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| Duration of follow-up | Treatment Pattern [a] | N [b] | CFA clearance [c] | Proportion of CFA-negative subjects at the end of follow-up |
|---|---|---|---|---|
| 1 year | 0 | 5 | 2 | 40.0% |
| | 1 | 30 | 6 | 20.0% |
| | 2 | 54 | 15 | 27.8% |
| | **Total** | 89 | 23 | 25.8% |
| 2 years | 0 2 | 2 | 1 | 50.0% |
| | 1 0 | 1 | 1 | 100% |
| | 1 1 | 8 | 5 | 62.5% |
| | 1 2 | 12 | 8 | 66.7% |
| | 2 1 | 15 | 5 | 33.3% |
| | 2 2 | 52 | 30 | 57.7% |
| | **Total** | 90 | 50 | 55.6% |
| 3 years | 0 0 0 | 2 | 1 | 50.0% |
| | 0 2 0 | 1 | 1 | 100% |
| | 0 2 1 | 1 | 1 | 100% |
| | 1 0 2 | 1 | 0 | 0% |
| | 1 1 0 | 2 | 2 | 100% |
| | 1 1 2 | 1 | 1 | 100% |
| | 1 2 0 | 3 | 3 | 100% |
| | 1 2 1 | 2 | 1 | 50.0% |
| | 1 2 2 | 11 | 5 | 45.5% |
| | 2 0 1 | 1 | 0 | 0% |
| | 2 0 2 | 1 | 0 | 0% |
| | 2 1 1 | 3 | 1 | 33.3% |
| | 2 1 2 | 13 | 6 | 46.1% |
| | 2 2 0 | 14 | 14 | 100% |
| | 2 2 1 | 6 | 3 | 50% |
| | 2 2 2 | 88 | 51 | 57.9% |
| | **Total** | 150 | 90 | 60.0% |
| 4 years | 0 1 2 0 | 1 | 1 | 100% |
| | 0 2 1 2 | 1 | 1 | 100% |
| | 1 1 2 0 | 1 | 1 | 100% |
| | 1 1 2 2 | 1 | 0 | 0% |
| | 1 2 0 0 | 1 | 1 | 100% |
| | 1 2 2 2 | 6 | 2 | 33.3% |
| | 2 0 2 1 | 1 | 0 | 0% |
| | 2 1 2 0 | 1 | 1 | 100% |
| | 2 1 2 2 | 1 | 1 | 100% |
| | 2 2 0 0 | 4 | 4 | 100% |
| | 2 2 1 0 | 2 | 2 | 100% |
| | 2 2 2 0 | 8 | 8 | 100% |
| | 2 2 2 1 | 3 | 2 | 66.7% |
| | 2 2 2 2 | 34 | 16 | 47.0% |
| | **Total** | 65 | 40 | 61.5% |
| **Total** | | 394 | 203 | 51.5% |

**Supplementary material 1**. Proportion of subjects who were CFA-negative at the end of their follow-up according to the number of albendazole doses received during each year of follow-up ("treatment pattern")

[a] the first digit corresponds to the number of treatment received during the first year of follow-up, the second digit corresponds to the number of treatment received during the second year of follow-up, etc.

[b] numbers of subjects corresponding to each pattern

[c] numbers of subjects who experienced CFA negativation during the last year of follow-up.

| Variables | Categories | Adjusted coefficients | 95% CI [a] | *P* |
|---|---|---|---|---|
| Sex | Female | Ref. | | |
| | Male | -47.1 | -147.5 – 53.3 | .358 |
| Age | 5 – 17 years | Ref. | | |
| | 18 – 30 years | -68.5 | -206.4 – 69.5 | .331 |
| | 31 – 45 years | 28.6 | -103.7 – 161.0 | .672 |
| | ≥ 46 years | -60.4 | -191.8 – 70.9 | . 367 |
| Initial CFA score | 1 | Ref. | | |
| | 2 | -43.7 | -247.2 – 159.8 | .674 |
| | 3 | 56.1 | -145.0 – 257.3 | .584 |
| Initial MFD | 1 – 200 Mf/mL | Ref. | | |
| | > 200 Mf/mL | 175.8 | 81.6 – 270.0 | < .001 |
| Bednets | No | Ref. | | |
| | Yes | -11.0 | -100.9 – 78.9 | .810 |
| Fishing | No | Ref. | | |
| | Yes | 16.3 | -94.3 – 126.9 | .773 |
| Sleep outside | No | Ref. | | |
| | Yes | -31.7 | -143.2 – 79.7 | .577 |
| Annual treatment | 0 dose | Ref. | | |
| | 1 dose | -78.7 | -455.4 – 297.9 | .682 |
| | 2 doses | -62.5 | -427.3 – 302.3 | .737 |
| FP1 (Time)[b] | Continuous | -286.9 | -2221.3 – 1647.5 | .771 |
| FP2 (Time)[c] | Continuous | 128.8 | -806.3 – 1063.9 | .787 |
| Annual treatment interacted with FP1 (Time) | 0 dose | Ref. | | |
| | 1 dose | -654.1 | -2698.4 – 1390.3 | .531 |
| | 2 doses | -450.9 | -2417.8 – 1515.9 | .653 |
| Annual treatment interacted with FP2 (Time) | 0 dose | Ref. | | |
| | 1 dose | 392.7 | -631.7 – 1417.1 | .452 |
| | 2 doses | 132.6 | -841.5 – 1106.7 | .790 |

**Supplementary material 2. Model results for the evolution of Mf density (MFD) with time as a fractional polynomial of order 2.**

[a] 95% confidence intervals

[b] the transformation for the Fractional polynomial 1 is: log(time) - .7406723224

[c] the transformation for the Fractional polynomial 2 is: $\log(\text{time})^2$ - .5485954892

| Bed net usage | Treatment adherence N (%) | | | Total |
|---|---|---|---|---|
| | 0 dose in the year | 1 dose in the year | 2 doses in the year | |
| **No** | 60 (55.6%) | 109 (37.5%) | 253 (30.8%) | 422 (34.6%) |
| **Yes** | 48 (44.4%) | 182 (62.5%) | 569 (69.2%) | 799 (65.4%) |
| **Total** | 108 | 291 | 822 | 1221 |

Cuzick test: Z = 4.992, $P = 0.0001$

Spearman coefficient between bed net usage (yes/no) and treatment adherence (0, 1 or 2 doses) for all observations: 0.129 ($P < 0.0001$)

**Supplementary material 3**. Relationship between bed nets use and treatment adherence for all observations included in parametric survival model on CFA clearance (N: number of observations, %: percentage of observations according to bed net usage)

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Parasites & Vectors

RESEARCH Open Access

# A strong effect of individual compliance with mass drug administration for lymphatic filariasis on sustained clearance of soil-transmitted helminth infections

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## Abstract

**Background:** The impact of semiannual mass drug administration (MDA) with albendazole (ALB; 400 mg) alone on lymphatic filariasis (LF) and soil-transmitted helminth (STH) infections was assessed during two trials conducted from 2012 to 2018 in the Republic of Congo and the Democratic Republic of Congo. The collected data were analyzed to evaluate the effect of compliance with ALB treatment on STH infections.

**Methods:** STH infections were diagnosed with duplicate Kato-Katz thick smears and the results are reported as eggs per gram of stool. All subjects with at least two STH infection assessments were included in the analyses. We used parametric survival models to assess the influence of compliance with ALB treatment on the probability of (i) achieving sustained clearance of an STH infection, and (ii) acquiring an STH infection during the follow-up.

**Results:** Out of 2658 subjects included in the trials, data on 202 participants (701 person-years; PY) with hookworm infection, 211 (651 PY) with *Ascaris lumbricoides* infection and 270 (1013 PY) with *Trichuris trichiura* infection were available to calculate the probability of achieving sustained clearance of infection. The effect of ALB was dose related for all three STH. For hookworm, the time required for sustained clearance was longer (4.3 years, $P < 0.001$) for participants who took zero doses per year and shorter (3.4 years, $P = 0.112$) for participants who took two doses per year compared to those who took one dose per year (3.7 years). For *Ascaris*, the time required to obtain sustained clearance followed the same pattern: 6.1 years ($P < 0.001$) and 3.2 years ($P = 0.004$) *vs* 3.6 years for, zero, two and one dose per year, respectively. For *Trichuris*, less time was required for sustained clearance (4.2 years, $P < 0.001$) for fully compliant participants, i.e. those who took two doses per year, than for those who only took one dose per year (5.0 years). ALB was more effective in achieving sustained clearance of STH infection in subjects with light baseline infection intensities compared to those with higher egg counts.

**Conclusion:** Our results illustrate the importance of MDA compliance at the level of the individual with respect to the STH benefit provided by semiannual ALB MDA, which is used for the elimination of LF in Central Africa.

**Keywords:** Soil-transmitted helminths, Albendazole, Parametric survival analysis, Treatment adherence, Mass drug administration

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## Background

Soil-transmitted helminth (STH) infections are among the most common infections in the world and affect poor and disadvantaged communities, particularly in sub-Saharan Africa, Southeast Asia and Latin America. In 2010, it was estimated that 438.9 million people were infected with hookworm, 819.0 million with *Ascaris lumbricoides* and 464.6 million with *Trichuris trichiura* [1]. The current strategy used to control STH infections is to conduct periodical deworming campaigns without individual diagnosis that target at-risk populations in endemic areas (preschool children, school-age children, women of reproductive age and adults in certain high-risk occupations). The World Health Organization (WHO) recommends the use of benzimidazoles: albendazole (ALB; 400 mg) or mebendazole (500 mg). Because of logistic and cost constraints, preventive chemotherapy campaigns for STH are usually conducted once or twice per year, depending on the initial prevalence of infection with any STH [2]. However, the optimal frequency of administration to maximize impact remains a topic of discussion [3]. In this study, we had the opportunity to assess the individual effect of a semiannual ALB treatment on STH infections. The data originate from two community trials that were designed to evaluate the effect of mass drug administration (MDA) with semiannual ALB on lymphatic filariasis (LF) in two countries in central Africa. The first study was conducted in a village in the Republic of the Congo (Congo), where *A. lumbricoides* infection prevalence decreased significantly from 56.5% to 12.9% and *T. trichiura* infection prevalence decreased significantly from 78.6% to 59.4% after 7 rounds of ALB MDA with global treatment adherence between 83 and 90% [4]. The second study took place in two contiguous villages in the Democratic Republic of the Congo (DRC), where hookworm infection prevalence decreased significantly from 58.6% to 21.2% after eight rounds of ALB MDA with a global treatment adherence of between 56 and 88%; *Ascaris* and *Trichuris* infection prevalences also decreased (from 14.0% to 1,6% and from 4.1% to 2.9%, respectively) [5]. The results of these two trials are consistent with those previously observed regarding ALB efficacy: according to a meta-analysis of more than 50 clinical trials, ALB has very good efficacy for clearing hookworm infections, good efficacy for *Ascaris* infections, but only moderate efficacy for *Trichuris* infections [6].

Some studies have shown that semiannual ALB MDA is highly effective for reducing STH prevalence at the community level [4, 5, 7–10]. However, considerable heterogeneity has been observed at the individual level, and no prior studies have examined the impact of the compliance of individuals with MDA on their STH infections. For example, in one study, infections cleared in some individuals after a single treatment, while infections were still present in others (due to persistence or reinfection) after eight rounds of semiannual MDA [11]. In the present study, we used longitudinal treatment and parasitology data collected between 2012 and 2018 from two community MDA studies to assess relationships between MDA compliance by individuals with STH infections and their subsequent infection status. The results indicate a clear link between MDA compliance by individuals and sustained clearance of STH infections.

## Methods

### Study population

The design of the MDA studies has been described elsewhere [4, 5]. In Congo, the study was conducted from 2012 to 2015 in Seke-Pembe, a village located in Mabombo health district (Bouenza division). In the DRC, the study site consisted of two neighboring villages (Mbunkimi and Misay) located in Kwilu province, and the trial took place from 2014 to 2018. Study participants were tested for STH infections at baseline and then annually. No deworming program had ever been conducted in the two areas prior to our trials. Both studies were approved by ethics committees and administrative authorities in the respective countries. Adult participants signed an informed consent form. Participants aged < 18 years were enrolled only after verbal assent and if one parent signed a consent form.

During the course of the two trials, a total of 2658 individuals were examined at least once for LF, and a total of 1573 provided stool samples at least once.

### Assessment of STH infections

Annual parasitological assessments were performed for participants > 5 years of age. STH infections were detected by microscopic examination of stool specimens. Participants were given a 50-mL plastic stool container and asked to collect a sample of their stool in the morning. The stool specimens were collected and stored in cooling boxes and shipped within 6 h to the laboratory, where they were immediately processed or stored overnight at 6 °C. Two thick smears were prepared according to the Kato-Katz method for each stool sample [12]. Thick smears were examined by microscopy at 40× magnification, and the slides prepared from each sample were examined by two different microscopists. The arithmetic mean egg count from the two slides was calculated, and the results are expressed for each species as eggs per gram of stool (EPG).

### Drug distribution and assessment of treatment adherence

All participants were offered treatment with one ALB tablet (400 mg) that was swallowed under the direct observation of study staff. All inhabitants who had not participated in the parasitological survey or who missed testing (due to absence or refusal) were later visited at home and offered ALB treatment. All treatments were provided under the supervision of a local healthcare worker who was also responsible for conducting a population census before each semiannual MDA. Every treatment was recorded in a drug treatment register. In addition, participants were asked whether they had received ALB during the previous MDA campaign (6 months earlier) during annual parasitological surveys. Therefore, for each annual parasitological assessment, we determined if each participant had taken two, one or zero ALB treatments since the last parasitological assessment.

### Statistical analysis

The primary endpoint (i.e. event of interest) for the study was conversion in STH infection(s). This was considered separately for conversion from a positive to a negative test during follow-up (defined as the sustained clearance of infection analysis) and for conversion from negative to positive (defined as the incident infection analysis).

The sustained clearance analysis included all individuals who were positive for *A. lumbricoides*, *T. trichiura* or hookworm at the time of their first test (which was not necessarily performed during the year when the trial started at the site) who also had at least one subsequent stool sample tested for STH. Therefore, individuals who were negative at baseline and remained negative throughout their follow-up were not included in the analyses (representing 59.1%, 47.8% and 37.6% of the population with at least one follow-up test for hookworm, *Ascaris* and *Trichuris*, respectively). The numbers of excluded individuals are provided in Additional file 1: Table S1. A sensitivity analysis including data on all the participants who were positive at the time of their first test but who had become negative by their follow-up, regardless of whether they subsequently became positive again, is provided in Additional file 1: Table S2.

The incident infection analysis included all individuals who were negative for *A. lumbricoides*, *T. trichiura* or hookworm at the time of their first stool test who had a positive stool test at a later time point (regardless of whether they subsequently became negative again).

We used survival analysis methods for the sustained clearance and incident infection analyses. The start date for the survival analysis was the first visit (index date) with a positive STH test for the sustained clearance of infection analysis or a negative STH test for the incident infection analysis. Individual observations were censored at the end of the follow-up or at the date of the event (date of the annual parasitological survey). Each participant's data were considered for calculation of cumulative person-years (PY) in the survival analysis.

We considered the following non-time-varying covariates for each STH analysis: sex and initial EPG intensity (categorized according to WHO guidelines [13]). We also considered the following time-varying covariates: age (categorized according to interquartile and median values—5–8, 9–12, 13–30 and $\geq$ 31 years old); and the number of ALB tablets taken during the previous year (0, 1 or 2).

Univariate analysis of infection status conversion rates was conducted using Mantel–Haenszel tests. We used parametric survival models with accelerated failure time to estimate the influence of time-varying variables on infection status conversion (time-to-event) [14, 15]. These models allow longitudinal analyses with time-varying variables; they are more informative and provide time ratios that enable prediction of mean time until an event occurs (either sustained clearance or incident infection events).

Several time distributions that do not require meeting the proportional risk assumption were tested according to the Akaike information criterion (AIC). Random effects at the household and at the village level were assessed in all survival models, and significance was assessed using the results of likelihood ratio tests. Significant random effects were retained in the models. Results are presented as time ratios with 95% confidence intervals (CI). Time ratios represent time differences to event (individual infection status conversion) according to the reference category. Sociodemographic data, occupation, initial infection intensity and the number of ALB tablets taken per year were included in the *Ascaris*, *Trichuris* and hookworm infection status conversion survival models. For the *Trichuris* model, the variable Numbers of ALB tablets taken per year had only two categories (1 or 2) because only 1 PY contributed to the zero-dose category. Predicted average times to infection status conversions were estimated using the command margins in STATA v.15.1 software (StatCorps, College Station, TX) [16].

For individuals for whom sustained clearance of infection was achieved, mixed models with random effects were used to describe changes in EPG according to time, treatment history and sociodemographic information for each STH infection. Several transformations (linear, quadratic, first-order fractional polynomials and second-order fractional polynomials) were tested for the time variable, and selection was made according to AIC. Random effects at village level were considered for the parametric survival analysis and mixed models for changes in

EPG. Lastly, the significance of relevant interaction terms was assessed (age and sex, age and initial infection intensity, age and number of ALB treatments taken, sex and initial infection intensity, sex and number of ALB treatments taken) for all models. All analyses were performed using STATA v.15.1 software.

## Results

### Study participants

For the sustained clearance model, repeated observations were made for the hookworm infection analysis for 202 of 2658 participants enrolled in the studies (7.6%), with 701 PY of observations. Stool examinations were negative for hookworm for 135 of these participants (66.8% of individuals diagnosed with hookworm infection at their first parasitological exam) during the course of the study, which remained the case until their last follow-up visit. For *Ascaris*, there were longitudinal data for 211 (7.9%) participants for analysis, with 681 PY of observations. In 172 of these participants (81.5% of individuals with *Ascaris* at their first parasitological exam), stool samples became negative during the course of the study and remained so until their last follow-up visit. For *Trichuris*, there were baseline and follow-up data for 270 participants (10.2% of all study participants) for the analysis, with 1,019 PY of observations. Of these participants, 85 (31.5% of individuals with a *Trichuris* infection at their first parasitological exam) became negative during the course of the study and remained so until their last follow-up visit. The key infection survival data are summarized in Table 1 along with results of a bivariate analysis of co-factors. Of note, most individuals with hookworm infection lived in the DRC study site, whereas most individuals with *Ascaris* and *Trichuris* infections lived in the Congo study site.

For the incident infection model, out of 542 individuals negative for hookworm at baseline with at least one follow-up visit, 102 (220 PY) experienced an incident infection (18.8%). For *Ascaris*, out of 533 participants negative at baseline with at least one follow-up visit, 177 (727 PY) experienced an incident infection (33.2%). For *Trichuris*, out of 474 participants negative at baseline with at least one follow-up visit, 194 (772 PY) experienced an incident infection (40.9%).

### Bivariate analysis of sustained clearance of STH infection

The unadjusted sustained clearance rates for hookworm, *Ascaris* and *Trichuris* infections were 19.2, 25.2, and 8.4 events per 100 PY of observation, respectively (Table 1). Table 1 also shows sustained clearance rates adjusted for each covariable. Because the number of ALB tablets taken per year is a time-varying variable, it was not included in the sustained clearance rate calculations. For hookworm and *Ascaris*, the probability of sustained clearance was higher for females than for males. Older individuals converted to negative more often than children for all three STH infections. Despite low numbers of participants with heavy worm loads in the earlier years of the study, the initial intensity of infection was negatively correlated with the probability of sustained clearance for *Ascaris* and *Trichuris*. Finally, the probability of sustained clearance varied by village of residence.

### Parametric survival multivariate models for sustained clearance of STH infection

Parametric survival model results for sustained clearance are presented in Table 2. For the hookworm and *Ascaris* models, a log-normal distribution gave the best fit to the data, whereas a log-logistic distribution was a better fit for *Trichuris*. No significant interactions between the covariates were found. Household random effects were included for the *Trichuris* model [intraclass correlation coefficient (ICC) 12.2%, $P=0.002$]. Village random effects were included for the hookworm model (ICC 18.0%, $P<0.001$). Random effects were not significant for the *Ascaris* model. For hookworm, *Ascaris* and *Trichuris*, older individuals (> 30 years of age) achieved sustained clearance more rapidly than children aged 5–8 years (3.3 *vs* 5.7 years for hookworm; 3.1 *vs* 3.8 years for *Ascaris* and 4.1 *vs* 4.7 years for *Trichuris*, respectively). It took significantly longer for non-compliant individuals (zero doses per year) to achieve sustained clearance for *Ascaris* or hookworm than for individuals who took one dose per year [6.1 *vs* 3.6 years for *Ascaris* ($P<0.001$) and 4.3 *vs* 3.7 years for hookworm ($P<0.001$), respectively]. In addition, it took less time to achieve sustained clearance of *Ascaris* and *Trichuris* infection in individuals who were highly compliant with MDA (two doses per year) compared to individuals who took only one dose per year [3.2 *vs* 3.6 years for *Ascaris* ($P=0.004$) and 4.2 *vs* 5.0 years for *Trichuris* ($P<0.001$), respectively].

Figure 1 illustrates that, for all three STH infections, the predicted proportion of participants who experienced sustained clearance of infection was higher in highly compliant individuals and increased with the duration of follow-up. Figure 1 also shows that in individuals with low compliance, sustained clearance was observed after 2 and 3 years of follow-up for hookworm and *Ascaris* infection, respectively, whereas this started after 1 year in fully compliant individuals.

Regarding infection intensity, it took significantly longer to achieve sustained clearance in individuals with moderate to heavy initial infection intensity with *Trichuris* ($\geq 1000$ EPG) than in individuals with light infections ($<1000$ EPG) (4.2 *vs* 5.0 years, respectively). Baseline infection intensities did not significantly affect

**Table 1** Sustained clearance rates for hookworm, *Ascaris* and *Trichuris* infections

| Variables | Categories | Hookworm (202 individuals) | | | | | *Ascaris* (211 individuals) | | | | | *Trichuris* (270 individuals) | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | PY | No. of events | Rate[a] | 95% CI | *P* | PY | No. of events | Rate[a] | 95% CI | *P* | PY | No. of events | Rate[a] | 95% CI | *P* |
| | All participants | 701 | 135 | 19.2 | 16.2–22.8 | | 681 | 172 | 25.2 | 21.7–29.3 | | 1019 | 86 | 8.4 | 6.8–10.4 | |
| Sex | Male | 390 | 69 | 17.7 | 14.0–22.4 | 0.023 | 299 | 63 | 21.1 | 16.5–27.0 | 0.017 | 420 | 36 | 8.6 | 6.2–11.9 | 0.919 |
| | Female | 311 | 66 | 21.2 | 16.7–27.9 | | 382 | 109 | 28.5 | 23.7–34.4 | | 599 | 50 | 8.3 | 6.3–11.0 | |
| Age | 5–8 Years | 245 | 30 | 12.2 | 8.6–17.5 | 0.009 | 153 | 20 | 13.1 | 8.4–20.3 | 0.001 | 201 | 7 | 3.5 | 1.7–7.3 | < 0.001 |
| | 8–12 Years | 166 | 39 | 23.5 | 17.2–32.2 | | 189 | 46 | 24.3 | 18.2–32.5 | | 222 | 9 | 4.0 | 2.1–7.8 | |
| | 13–30 Years | 135 | 24 | 17.8 | 11.9–26.5 | | 117 | 32 | 27.3 | 19.3–38.7 | | 209 | 16 | 7.6 | 4.7–12.5 | |
| | ≥ 31 Years and more | 155 | 42 | 27.1 | 20.0–36.6 | | 222 | 74 | 33.3 | 26.5–41.9 | | 387 | 54 | 13.9 | 10.7–18.2 | |
| Initial infection intensity[b] | Light | 575 | 131 | 20.0 | 16.8–23.7 | 0.059 | 269 | 77 | 28.6 | 22.9–35.8 | 0.022 | 634 | 74 | 11.7 | 9.3–14.7 | < 0.001 |
| | Moderate | 29 | 4 | 13.8 | 5.2–36.5 | | 374 | 87 | 23.3 | 18.8–28.7 | | 369 | 11 | 3.0 | 1.6–5.4 | |
| | Heavy | 16 | 0 | 0 | N/A | | 38 | 8 | 21.0 | 10.5–42.1 | | 16 | 1 | 6.2 | 0.9–44.4 | |
| Village | Misay | 323 | 61 | 18.9 | 14.7–24.3 | 0.001 | 44 | 8 | 18.2 | 9.1–36.3 | 0.247 | 3 | 1 | N/A | N/A | |
| | Mbunkimi | 336 | 56 | 16.7 | 12.8–21.6 | | 41 | 13 | 31.7 | 18.4–54.6 | | 20 | 4 | N/A | N/A | |
| | Seke Pembe | 42 | 18 | 42.8 | 27.0–68.0 | | 596 | 151 | 25.3 | 21.6–29.7 | | 994 | 80 | 8.0 | 6.5–10.0 | |

*P* is calculated from significance tests using the Mantel–Haenszel method based on stratified rate ratios

*PY* Person-years, *CI* confidence interval

[a] Infection status conversion rate (for 100 PY)

[b] According to World Health Organization (WHO) guidelines: for hookworm, 1–1999 (light), 2000–3999 (moderate), > 4000 eggs per gram of stool (EPG) (heavy); for *Ascaris*, 1–4999 (light), 5000–49,999 (moderate), > 49,999 EPG (heavy); for *Trichuris*, 1–999 (light), 1000–9999 (moderate), > 10,000 EPG (heavy)

**Table 2** Results of parametric survival models for sustained clearance of hookworm, *Ascaris* and *Trichuris* infections (with random effects)

| Variables | Categories | Hookworm | | *Ascaris* | | *Trichuris* | |
|---|---|---|---|---|---|---|---|
| | | TR/95% CI[a] | *P* | TR/95% CI[a] | *P* | TR/95% CI[a] | *P* |
| Sex | Female | Ref. | | Ref. | | Ref. | |
| | Male | 1.10 (1.01, 1.19) | 0.020 | 1.11 (1.02, 1.21) | 0.013 | 0.99 (0.92, 1.06) | 0.807 |
| Age | 5–8 Years | Ref. | | Ref. | | Ref. | |
| | 8–12 Years | 0.93 (0.83, 1.04) | 0.223 | 0.95 (0.86, 1.08) | 0.398 | 1.06 (0.91, 1.23) | 0.452 |
| | 13–30 Years | 0.87 (0.77, 0.98) | 0.019 | 0.86 (0.75, 1.00) | 0.046 | 0.96 (0.83, 1.10) | 0.557 |
| | More than 30 years | 0.79 (0.70, 0.88) | < 0.001 | 0.81 (0.71, 0.92) | 0.001 | 0.88 (0.77, 0.99) | 0.049 |
| Initial infection intensity[b] | Light | Ref. | | Ref. | | Ref. | |
| | Moderate to heavy | 1.20 (0.98, 1.46) | 0.072 | 1.06 (0.97, 1.16) | 0.170 | 1.18 (1.07, 1.31) | < 0.001 |
| Treatment | Zero dose per year | 1.16 (1.00, 1.35) | < 0.001 | 1.74 (1.28, 2.38) | < 0.001 | Not calculable | |
| | One dose per year | Ref. | | Ref. | | Ref. | |
| | Two doses per year | 0.91 (0.81, 1.02) | 0.112 | 0.87 (0.79, 0.95) | 0.004 | 0.84 (0.77, 0.91) | < 0.001 |
| Random effects | | Village | < 0.001 | Not included | | Household | 0.002 |
| | ICC | 18.0% | | | | 12.2% | |
| Model | Distribution | Log normal | | Log normal | | Log logistic | |
| | AIC | 464.1 | | 515.9 | | 410.2 | |
| | Log likelihood | − 222.0 | | − 249.0 | | − 196.1 | |

*Ref.* Reference, *ICC* intraclass correlation coefficient, *AIC* Akaike information criterion; for other abbreviations, see Table 1

[a] Adjusted time ratio (*TR*)/95% confidence intervals (*CI*). For example, for the *Ascaris* model, compared to a female (TR = 1), it took 11% (TR = 1.11) more time to achieve sustained clearance in a male

[b] According to WHO guidelines: for hookworm, 1–1999 (light), > 2000 EPG (moderate to heavy); for *Ascaris*, 1–4999 (light), > 5000 EPG (moderate to heavy); for *Trichuris*, 1–999 (light), > 1000 EPG (moderate to heavy)

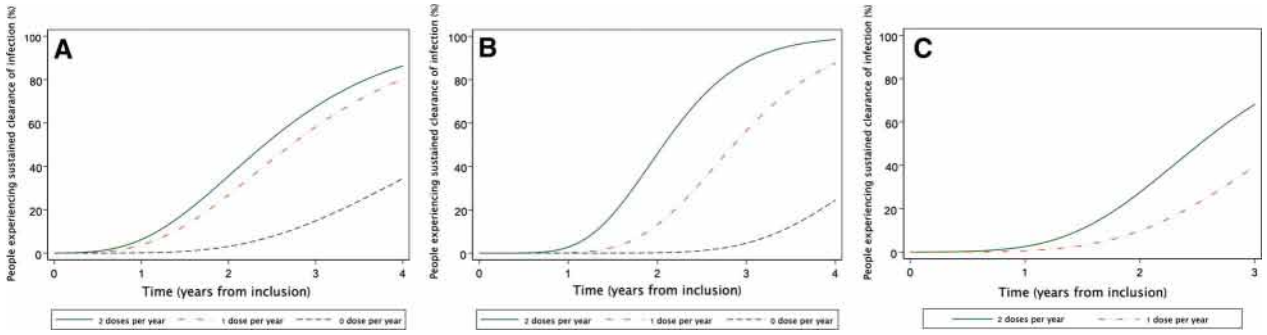


**Fig. 1** **a–c** The influence of compliance with albendazole treatment on soil-transmitted helminth infection status in study participants with prior infections. **a** Hookworm, **b** *Ascaris*, **c** *Trichuris*

the time to sustained clearance for *Ascaris* or hookworm infections.

### Parametric survival multivariate models of STH incident infection

Parametric survival model results for incident infection are presented in Table 3. A log-logistic distribution gave the best fit for data in the hookworm and *Trichuris* models; a log-normal distribution provided the best fit for the *Ascaris* model. No interactions between the covariates were found. Household random effects were included for the *Trichuris* model (ICC 42.9%, $P < 0.001$). Village random effects were included for the *Ascaris* models (ICC 45.7%, $P < 0.001$) and hookworm model (ICC 31.4%, $P < 0.001$). For hookworm and *Trichuris*, males acquired infection significantly more slowly than females. For the *Trichuris* model, older individuals (> 13 years) acquired infection significantly faster than individuals

**Table 3** Results of parametric survival models for incident hookworm, *Ascaris* and *Trichuris* infections (with random effects)

| Variables | Categories | Hookworm | | *Ascaris* | | *Trichuris* | |
|---|---|---|---|---|---|---|---|
| | | TR/95% CI | *P* | TR/95% CI | *P* | TR/95% CI | *P* |
| Sex | Female | Ref. | | Ref. | | Ref. | |
| | Male | 1.39 (1.11, 1.73) | 0.004 | 1.10 (0.94, 1.19) | 0.234 | 1.22 (1.031, 1.44) | 0.018 |
| Age | 5–8 Years | Ref. | | Ref. | | Ref. | |
| | 8–12 Years | 1.12 (0.87, 1.45) | 0.361 | 1.07 (0.88, 1.32) | 0.489 | 0.88 (0.73, 1.05) | 0.156 |
| | 13–30 Years | 1.02 (0.72, 1.46) | 0.872 | 1.13 (0.87, 1.46) | 0.364 | 0.62 (0.46, 0.84) | 0.002 |
| | More than 30 years | 0.95 (0.70, 1.28) | 0.738 | 1.11 (0.87, 1.41) | 0.412 | 0.53 (0.40, 0.70) | < 0.001 |
| Treatment | Zero doses per year | 0.79 (0.51, 1.22) | 0.290 | 0.62 (0.42, 0.91) | 0.001 | 0.78 (0.53, 1.13) | 0.190 |
| | One dose per year | Ref. | | Ref. | | Ref. | |
| | Two doses per year | 0.97 (0.70, 1.33) | 0.828 | 1.10 (0.80, 1.50) | 0.559 | 1.14 (0.85, 1.54) | 0.385 |
| Random effects | | Village | < 0.001 | Village | < 0.001 | Household | < 0.001 |
| | ICC | 31.4% | | 45.7% | | 42.9% | |
| Model | Distribution | Log logistic | | Log normal | | Log logistic | |
| | AIC | 505.5 | | 626.5 | | 718.4 | |
| | Log likelihood | − 243.7 | | − 304.2 | | − 350.2 | |
| Key survival data | Number of subjects | 102 | | 177 | | 194 | |
| | PY | 420 | | 727 | | 772 | |

For abbreviations, see Tables 1 and 2

aged 5–8 years. Non-compliant individuals (zero doses per year) acquired *Ascaris* infection significantly more quickly than individuals who took one dose of ALB per year.

### Changes in EPG over time

Linear transformation of time provided the best fit for the EPG data. No multilevel effects were included in these mixed models because neither the village nor the household effect was significant. Overall decreases in EPG were superior in individuals with better compliance (Table 4). For all three STH, the decline in EPG over time was slower in younger individuals, regardless of the number of ALB tablets taken per year.

According to the mixed model, decreases in hookworm EPG were not significantly different for individuals with different compliance patterns: —81.2 EPG/year (95% CI − 316.0–153.6) for one dose and − 197.1 EPG/year (CI 95% − 402.6–8.4) for two doses, compared with no treatment. Decreases in *Ascaris* EPG were significantly different between individuals who took zero and one dose per year (regression coefficient: − 7076.1 EPG/year, CI 95% − 13,103 to − 1048.9) and between those who took zero and two doses per year (regression coefficient: -6,207.3 EPG/year, CI 95% − 1.2196 to − 218.5). Finally, decreases in *Trichuris* EPG were not significantly different between individuals who took two doses per year (regression coefficient: -207.5 EPG/year, CI 95% − 603.2–188.3) and those who took one dose per year.

## Discussion

ALB is widely known to be effective for the treatment of STH infections. Community MDA with ALB can reduce *Ascaris* and hookworm prevalence, but the effects on *Trichuris* tend to be modest [6, 17]. This is the first longitudinal study to examine the effect of individual compliance with semiannual MDA with ALB alone, given for the elimination of LF, on STH infections. We found that good compliance with semiannual rounds of MDA resulted in shorter times to achieve sustained clearance of STH infections. This was likely due to a combination of curing existing infections and curing incident infections during the follow-up period. This dose-related effect was particularly strong for ascariasis. A similar pattern was observed for hookworm, although the difference between one and two doses per year was not statistically significant. This might indicate that reinfection is more rapid for hookworm than for *Ascaris*. Further studies are needed to validate this hypothesis. As individuals with *Trichuris* infection in this study were generally compliant with MDA, we were unable to compare times to infection status conversion based on ALB intake. It is interesting that some non-compliant individuals with hookworm or *Ascaris* infections achieved sustained clearance, and that this phenomenon was more common in the later years of the study, as shown in Fig. 1. Although some spontaneous loss of infection was expected, particularly for light infections, an increase in sustained clearance events in later years might have been due to a reduced force of

**Table 4** Mixed model results for the evolution of EPG intensity

| Variables | Categories | Hookworm | | *Ascaris* | | *Trichuris* | |
|---|---|---|---|---|---|---|---|
| | | Coeff./95% CI[a] | *P* | Coeff./95% CI[a] | *P* | Coeff./95% CI[a] | *P* |
| Sex | Female | Ref. | | Ref. | | Ref. | |
| | Male | 65.8 (− 34.4, 166.0) | 0.199 | 1056.7 (− 1115.9, 3229.2) | 0.340 | 234.2 (− 139.1, 607.5) | 0.219 |
| Age | 5–8 Years | Ref. | | Ref. | | Ref. | |
| | 8–12 Years | 32.6 (− 122.6, 187.8) | 0.681 | − 4540.2 (− 7576.5, − 1504.0) | 0.003 | 126.1 (− 439.3, 691.6) | 0.662 |
| | 13–30 Years | − 128.0 (− 274.1, 18.0) | 0.086 | − 6432.5( − 9910.3, − 2954.8) | 0.0001 | − 333.9 ( − 929.6, 261.7) | 0.272 |
| | More than 30 years | − 107.1 (− 251.4, 37.1) | 0.088 | − 8030.3 (− 11035, − 5024.5) | < 0.001 | − 498.6 (− 1179.8, − 162.3) | 0.010 |
| Initial infection intensity[b] | Light | Ref. | | Ref. | | Ref. | |
| | Moderate | 1155.4 (929.3, 1383, 5) | < 0.001 | 8727.8 (6302.6, 11153) | < 0.001 | 1061.2 (653.1, 1469.4) | < 0.001 |
| | Heavy | 3171.6 (2875.8, 3467.3) | < 0.001 | 33, 843 (28637, 39050) | < 0.001 | 6886.7 (5518.5, 8254.9) | < 0.001 |
| Time[c] | Continuous | − 58.2 (− 263.2, 146.6) | 0.577 | 1631.5 (− 4174.5, 7437.4) | 0.582 | − 91.9 (− 378.8, 195.0) | 0.530 |
| Annual treatment interaction with time[c] | Zero doses | Ref. | | Ref. | | Not calculable | |
| | One dose | − 81.2 (− 316.0, 153.6) | 0.498 | − 7076.1 (− 13,103, − 1048.9) | 0.021 | Ref. | |
| | Two doses | − 197.1 (− 402.6, 8.4) | 0.060 | − 6207.3 (− 12,196, − 218.5) | 0.042 | − 207.5 (− 603.2, 188.3) | 0.304 |
| Study site | Bandundu | Ref. | | Ref. | | Ref. | |
| | Seke Pembe | − 196.9 (− 427.8, 34.0) | 0.095 | 2596.8 (− 1004.8, 6198.3) | 0.158 | 721.0 (− 410.1, 1852.1) | 0.212 |
| Intercept at baseline | Zero doses | 387.4 (− 478.5, 1253.2) | 0.381 | − 7884 − 33,495, 17,725.7) | 0.546 | Not calculable | |
| | One dose | 272.7 (− 636.0, 1181.4) | 0.556 | 22,598 (− 3365.0, 48,561) | 0.088 | − 199.5 (− 170.9, 1305.9) | 0.795 |
| | Two doses | 404.0 (− 439.6, 1247.7) | 0.348 | 22,888 (− 2750.4, 48,527) | 0.080 | 936.6 (− 192.0, 2065.1) | 0.104 |

[a] Adjusted regression coefficient (*Coeff.*)/95% CI

[b] According to WHO guidelines: for hookworm, 1–1999 (light), 2000–3999 (moderate), > 4000 EPG (heavy); for *Ascaris*, 1–4999 (light), 5000–49,999 (moderate), > 49,999 EPG (heavy); for *Trichuris*, 1–999 (light), 1000–9999 (moderate), > 10,000 EPG (heavy)

[c] Interpretation of the interaction variable: for the hookworm model, all else being equal, each participant's EPG decreased by 58.2 each year; and all else being equal, participants taking one dose and two doses per year, as compared to zero doses, showed a decrease in their EPG by 81.2 and 197.1 EPG per year, respectively

infection in study communities (due to a "herd treatment effect") as a result of MDA.

The use of random effects at the household level improved the parametric survival model for *Trichuris*. Continued infection in households due to non-compliance or shared poor sanitation might increase the risk of reinfection for other household members who do comply with treatment. This would tend to increase the mean time for the sustained clearance of infection. Additional studies will be needed to test this hypothesis and to understand why the same household effect was not seen with hookworm or *Ascaris* infection.

It is interesting that incident infections were observed for all three STH infections during the course of the study despite community MDA. While most of these were probably true incidence events, it is likely that some of them were examples of pseudo-incidence, which may occur if light infections are not detected in prior stool samples.

Based on our analysis, good compliance with MDA was most effective for preventing *Ascaris* infections. This was because random household effects were significant for the *Trichuris* incidence model and for the hookworm incidence model. This means that a fraction of the incident infections for hookworm and *Trichuris* were due to random household effects regardless of MDA compliance. Random effects set at the village level were significant for the hookworm sustained clearance model and the *Ascaris* incident infection model. This suggests that a significant proportion of these status conversions were due to common village-specific effects related to sanitation and/or infection-promoting behavior.

Higher infection intensity was negatively associated with sustained clearance for all three STH, but this only

resulted in a significantly longer time to achieve sustained clearance for *Trichuris*. However, low baseline frequencies of moderate or high infection intensities for hookworm and *Ascaris* provided low statistical power for assessing the effect of infection intensity for these infections. For all models, a shorter time to sustained clearance was associated with older age. This is consistent with higher reinfection rates in children due to their behavior and exposure (e.g. frequent close contact with other children, walking barefoot outdoors, and hand to soil to mouth) relative to those of older people.

We used mixed models to study changes in infection intensity (EPG) over time according to the number of ALB tablets taken per year for each STH. Only the *Ascaris* model showed a greater decrease in EPG for compliant participants. No significant differences were found for EPG decrease and ALB compliance in the hookworm and *Trichuris* models. This was probably due to a lack of power because EPG trended down in regression models as compliance increased.

One limitation of this study is that results may have been influenced by prevalence or participation bias. We believe that prevalence bias is unlikely to have impacted our results because fewer than 20% of the study population had taken ALB prior to our study. On the other hand, participation bias cannot be excluded because participants with a high participation frequency in our study may have had different characteristics (including MDA adherence) than non-participants.

As previously reported, MDA and stool survey participation rates decreased over time in these community MDA studies [5]. This was probably due to study fatigue on the part of some community members. The results of this study that show the clear benefits of compliance with MDA may help social mobilization campaigns to increase initial and sustained MDA adherence by individuals and populations.

## Conclusions

This study demonstrated a clear dose–response relationship between individual MDA adherence with semiannual ALB and sustained clearance of STH infection over time. In other words, sustained clearance of STH infections was more likely to be achieved in individuals with good MDA adherence. These results should be used in social mobilization programs to publicize the fact that semiannual MDA with ALB for the elimination of LF also significantly contributes to the control of STH infections in individuals and communities.

**Abbreviations**

AIC: Akaike information criterion; ALB: Albendazole; CI: Confidence interval; DRC: Democratic Republic of the Congo; EPG: Eggs per gram of stool; ICC: Intraclass correlation coefficient; LF: Lymphatic filariasis; MDA: Mass drug administration; PY: Person-years; STH: Soil-transmitted helminth; WHO: World Health Organization.

## Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s13071-021-04814-2.

**Additional file 1: Table S1.** Individuals included or not included in the sustained clearance analysis. *DRC* Democratic Republic of the Congo, *Congo* Republic of the Congo. **Table S2.** Sensitivity analysis including data on participants who were positive at the time of their inclusion in the study and whose status sequentially changed to negative and to positive again during their follow-up.

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**Authors' contributions**

SDP, MB, CBC and GJW designed the study. NPAU, JPT, GKS, GJW, MB, CBC and SDP collected the data. JC, CBC and SDP analyzed the data. JC wrote the first manuscript draft. CBC, SDP, MB and GJW reviewed and edited the manuscript. All authors read and approved the final manuscript.

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**Availability of data and materials**

The datasets used and/or analyzed during the current study are available from the corresponding author on request.

## Declarations

**Ethics approval and consent to participate.**

This study is the follow-up of two trials approved by ethics committees [4, 5]. All the participants in the initial trials provided written informed consent.

**Consent for publication**

Not applicable.

**Competing interests**

The authors declare that they have no competing interests.

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## Publisher's Note

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Parasites & Vectors

RESEARCH Open Access

# Individuals living in an onchocerciasis focus and treated three-monthly with ivermectin develop fewer new onchocercal nodules than individuals treated annually

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## Abstract

**Background:** Little information is available on the effect of ivermectin on the third- and fourth-stage larvae of *Onchocerca volvulus*. To assess a possible prophylactic effect of ivermectin on this parasite, we compared the effects of different ivermectin regimens on the acquisition of onchocercal nodules.

**Methods:** We analyzed data from a controlled randomized clinical trial of ivermectin conducted in the Mbam Valley (Cameroon) between 1994 and 1998 in a cohort of onchocerciasis infected individuals. The number of nodules that appeared between the start and the end of the clinical trial was analyzed, using ANOVA and multivariable Poisson regressions, between four treatment arms: 150 µg/kg annually, 800 µg/kg annually, 150 µg/kg 3-monthly, and 800 µg/kg 3-monthly.

**Results:** The mean number of nodules that appeared during the trial was reduced by 17.7% in subjects treated 3-monthly compared to those treated annually (regardless of the dose). Poisson regression model, adjusting on subject's age and weight, initial number of nodules and intensity of *O. volvulus* infection in his village of residence, confirmed that the incidence of new nodules was reduced in 3-monthly treatment arms compared to annually treatment arms, and that the dosage of ivermectin does not seem to influence this effect. Furthermore, the number of newly acquired nodules was positively associated with the initial number of nodules. Analysis of disappearance of nodules did not show any significant difference between the treatment groups.

**Conclusions:** To our knowledge, these results suggest for the first time in humans, that ivermectin has a partial prophylactic effect on *O. volvulus*. Three-monthly treatment seems more effective than annual treatment to prevent the appearance of nodules.

**Keywords:** Onchocerciasis, *Onchocerca volvulus*, Nodules, Onchocercoma, Ivermectin, Cameroon

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## Background

Human onchocerciasis, also called "river blindness", is a neglected tropical disease (NTD) caused by the filarial nematode *Onchocerca volvulus*. In 1995, the World Health Organization (WHO) launched the African Programme for Onchocerciasis Control (APOC), which was mainly based on mass drug administration (MDA) of ivermectin (IVM). IVM, which is usually given annually at the standard dose of 150 μg/kg of body weight, has a direct effect on the microfilariae (mf) present in the skin (microfilaricidal effect) and prevents the release of new mf by the adult female worms for several months (embryostatic effect). However, the effect of IVM on the viability of the adult worms (macrofilaricidal effect) is considered as moderate and treatments have to be repeated every year (or at shorter intervals) to maintain skin microfilarial densities (MFD) at low levels not associated with clinical manifestations. Besides this, little information is available on the effect of IVM on the third- and fourth-stage larvae (L3s and L4s) which develop to the adult stage during the first months following the bite of an infective blackfly, and on the immature adults. The effect on these L3s, L4s, and immature adults, which would prevent the development up to the stage of fecund adult worms releasing mf, has been called causal prophylaxis, or suppressive effect [1]. We will use the term "prophylactic effect" throughout the text below.

Only four *in vivo* studies were conducted to evaluate the prophylactic effect of IVM on *Onchocerca* spp. The first one included 18 chimpanzees experimentally infected with *O. volvulus*. Six animals were treated with IVM (at 200 μg/kg) on the day of inoculation of the L3s, six were treated on day 28 and six were not treated. After having followed-up the development of infection by repeated skin biopsies for 30 months, the authors concluded that IVM could have a partial effect on the L3s (which live for about two to three days in the definitive host before molting to the L4 stage [2]), but no effect on the L4s [3]. The second study was conducted in an area of North Cameroon where the cattle parasite *O. ochengi* is endemic. Two groups of calves between two and eight weeks of age were treated monthly with subcutaneous IVM (Ivomec®) at either 200 μg/kg or 500 μg/kg for 21 months, and a third group was left untreated. Before each treatment, the animals were palpated for *O. ochengi* nodules and underwent a skin biopsy. The fact that none of the 15 treated calves developed adult worm infection, whereas five of the six control calves became infected led the authors to conclude that IVM had an effect on the L3s and L4s of *O. ochengi* [4]. The third study was conducted in an onchocerciasis hyperendemic focus (Mbam valley, Cameroon) and included human subjects with no *O. volvulus* mf in skin biopsies ("skin snips" taken with a 2 mm Holth punch). These patients were treated, just after the start of the high transmission period, with either a single oral dose of IVM (150 μg/kg) plus ferrous sulphate tablets, or the latter drug only. One year after, the incidence of *O. volvulus* microfilaridermia was 23.4% in the IVM group and 25.8% in the control group, and the mean MFD were similar in the two groups (2.2 and 2.7 mf per skin snip, respectively). The authors concluded that a single dose of IVM had no perceptible prophylactic effect in this highly endemic area [5]. The fourth study included calves (mean age: 9 weeks) naturally exposed to *O. ochengi* infection, and treated with IVM at monthly or 3-monthly intervals, or left untreated. After 22 months of exposure, 11 of the 14 control animals had acquired nodules (including ten with skin mf), two of the ten animals treated 3-monthly had nodules (but no skin mf), and none of the ten animals treated at monthly interval had acquired nodules [6]. These results suggest that 3-monthly treatment has a partial prophylactic effect on *O. ochengi*. Besides these trials, the effect of IVM on L3s and the L3-L4 molting process was supported by *in vitro* studies using *Onchocerca lienalis* [7, 8].

A double-blind randomized controlled trial aimed at assessing the potential macrofilaricidal effect of high (400–800 μg/kg) and/or more frequent (3-monthly) doses of IVM on *O. volvulus* was conducted in the Mbam valley (Cameroon) between 1994 and 1998. This effect was evaluated by the histologic examination of sections of nodules collected at the outset and at the end of the trial [9]. The proportion of dead female worms was found to be higher in the nodules collected from subjects treated 3-monthly than in those treated annually. During this trial, a careful examination for all palpable nodules was conducted at the outset of the trial, and during the nodulectomy round organized in 1997. The number and the location of each palpated nodule was noted on a standard chart. In the present paper, we present the results of statistical analyses performed on the number of nodules which had appeared or disappeared between the two examination rounds. Our main objective was to assess whether high doses or more frequent IVM treatment was associated with a lower number of new nodules (suggesting a prophylactic effect). Analyses were also performed on the number of nodules that had spontaneously disappeared.

## Methods

### Study population and subjects

The protocol of the trial has been described in detail elsewhere [9]. Briefly, it was conducted in the Bafia health district, located in the onchocerciasis hyperendemic focus of the Mbam Valley (Cameroon). Eligible subjects

were males aged 18–60 years-old in a good state of health, with no contra-indication to IVM, and who presented at least two palpable nodules at the outset of the trial.

#### Procedures

After having signed an informed consent form, subjects were randomly allocated to one of the four treatment groups receiving either 150 µg/kg annually (control group), or high dose (400 µg, then 800 µg/kg) annually, or 150 µg/kg 3-monthly or high dose 3-monthly. The pre-treatment nodulectomy round was performed in May-June 1994. Before the nodulectomy, each participant was carefully examined and the location of each palpable nodule was noted on a standard anatomic chart. The randomly selected nodule to remove during the operation was represented by a green dot on the chart, and all the others were noted as a red dot. After nodulectomy, each participant received a 150 µg/kg "clearing dose" of IVM to avoid the possibility of severe reactions developing in any patients subsequently taking their first dose on the high-dose regimen. The 3-year courses of treatment under investigation began in August 1994, 2–3 months after the clearing dose. A total of 643 subjects participated in this treatment round. The second round of nodulectomy was organized in August 1997. A total of 102 subjects was lost between August 1994 and August 1997 (24 deaths, 17 excluded on medical grounds and 61 subjects who moved away or were excluded because they missed one treatment round). The number of subjects participating in the second nodulectomy round was thus 541. Before the collection of the nodules, each patient was re-examined and the location of the nodules present was noted on the anatomic chart used in 1994. The nodules which had spontaneously disappeared in the interval were noted, and the location of the nodules which had appeared between 1994 and 1997 (thereafter called "new nodules") were noted by a blue dot. As these examinations had been performed just before the operation, an additional clinical examination was performed in November 1997, i.e. in a less time-constrained context, to confirm the results obtained three months before. This examination could be performed in 485 subjects and the statistical analyses were conducted on the latter.

#### Statistical analysis

Variables of interest were defined as (i) the number of nodules which appeared between 1994 and 1997 and (ii) the number of nodules which disappeared. For these two variables, we used the same statistical analysis plan (Fig. 1). First, we assessed the difference between people treated annually and people treated 3-monthly, regardless of the dose, using Student's t-test. Then, comparisons were performed between the four treatment arms using an ANOVA; Bonferroni test was subsequently used to assess which treatment arm(s) differed from the others. In case of an ANOVA-associated *P*-value $< 0.250$, we performed a multivariable analysis to assess the possible associations between appearance of new nodules and the following variables: treatment group as a categorical variable (first considering annual arms *versus* 3-monthly arms, then considering the four treatment arms), subject's age (continuous variable), subject's weight (continuous variable), initial number of palpable nodules (expressed sequentially as a continuous variable and as a categorical variable using the following categories: $< 5$ nodules and $\geq 5$ nodules), subject's initial microfilarial density (MFD, expressed as the number of mf per milligram of skin), and the intensity of *O. volvulus* infection in the participant's village of residence (proxy of exposure to infection). The individual MFD was calculated as the ratio of the sum of the number of mf found in both snips (taken from the left and right iliac crests) to the sum of the weights of each snip. In the analyses, the MFD was expressed as a categorical variable using the interquartile categories: $< 30$, 31–80, 81–170, and $> 170$ mf/mg. The intensity of *O. volvulus* infection in the participant's village of residence was expressed in three categories (low, moderate and high intensity) and was defined according to the community microfilarial load (CMFL) in the community measured during previous parasitological surveys in the community itself, or in neighbouring communities located at the same distance from the Mbam River. The CMFL, a classical indicator used to express the intensity of infection with *O. volvulus* in a community, corresponds to the Williams' geometric mean of the individual MFDs (here expressed as number of mf per skin snip) in all subjects examined (not only those skin snip positive) aged $\geq 20$ years-old [10]. The three categories used in the analyses correspond to CMFL $< 20$ mf/skin snip, 20–50 mf/skin snip and $\geq 50$ mf/skin snip. Multivariable logistic regression models were used with the dependent variable coded as a binary outcome (appearance of nodules *versus* no appearance of nodule). The reference group is defined as people with less than 5 nodules in the 150 µg/kg 3-monthly treatment group. Then, Poisson regression models were used, with the dependent variable recoded as 0, 1, 2, 3, 4 and $\geq 5$ new nodules. We constructed two models: one with the 4 treatment arms and one with the pooled annual and 3-monthly treatment arms, regardless of the dose. For all models, an interaction term between the treatment arm and the initial number of nodules was added to assess the mean number of new nodules according to these two variables simultaneously. For each regression model, we presented two procedures: a saturated model with all explicative variables, whatever

their *P*-values and a backward stepwise procedure with a *P*-value threshold of 0.150 for the explicative variables. We presented regression coefficients and their 95% confidence intervals (95% CI). The reference group is defined as people who had the lowest number of initial nodules (i.e. 2 nodules) in the 150 µg/kg annually group or in the annual treatment, depending on the model. According to this regression analysis, predictions were made using the *margins* and *marginsplot* commands.

All analyses were performed using the STATA v.15.1 software (StatCorps, LP, College Station, TX, USA).

## Results

### Baseline characteristics

Table 1 presents the baseline characteristics of the 485 study subjects as a whole and in each treatment arm. Before the first nodulectomy round, the participants of the four treatment groups were similar in terms of age (Kruskal–Wallis H-test: $\chi^2=1.36$, $df=3$, $P=0.714$), body weight (Kruskal–Wallis H-test: $\chi^2=4.98$, $df=3$, $P=0.172$), mean number of nodules (Kruskal–Wallis H-test: $\chi^2=3.49$, $df=3$, $P=0.349$), CMFL in the village of residence (Chi-square test: $\chi^2=4.77$, $df=6$, $P=0.574$) and MFD (Kruskal–Wallis H-test: $\chi^2=2.39$, $df=3$, $P=0.495$).

### Analysis of appearance of nodules between 1994 and 1997

The mean number of new nodules in the 237 subjects treated 3-monthly (1.85) was 17.7% lower than in the 248 subjects treated annually (2.25; t-test: $t_{(483)}=2.67$, $P=0.008$). The mean numbers of nodules that appeared between 1994 and 1997 in subjects of each treatment group are shown in Table 2. ANOVA indicated a statistically significant difference in the number of new nodules between the treatment groups (ANOVA: $F_{(3,\ 484)}=3.60$, $P=0.014$). Bonferroni correction showed that the number of new nodules was significantly lower in the group which had received 150 µg/kg 3-monthly than in the group treated with 800 µg/kg annually ($P=0.008$). No significant difference was found between the two groups treated annually ($P=1.000$), nor between the two groups treated 3-monthly ($P=0.871$), nor between the two groups treated with 150 µg/kg (annually *vs* 3-monthly) ($P=0.260$).

According to the logistic regression analysis (Table 3), the probability to develop new nodules did not differ

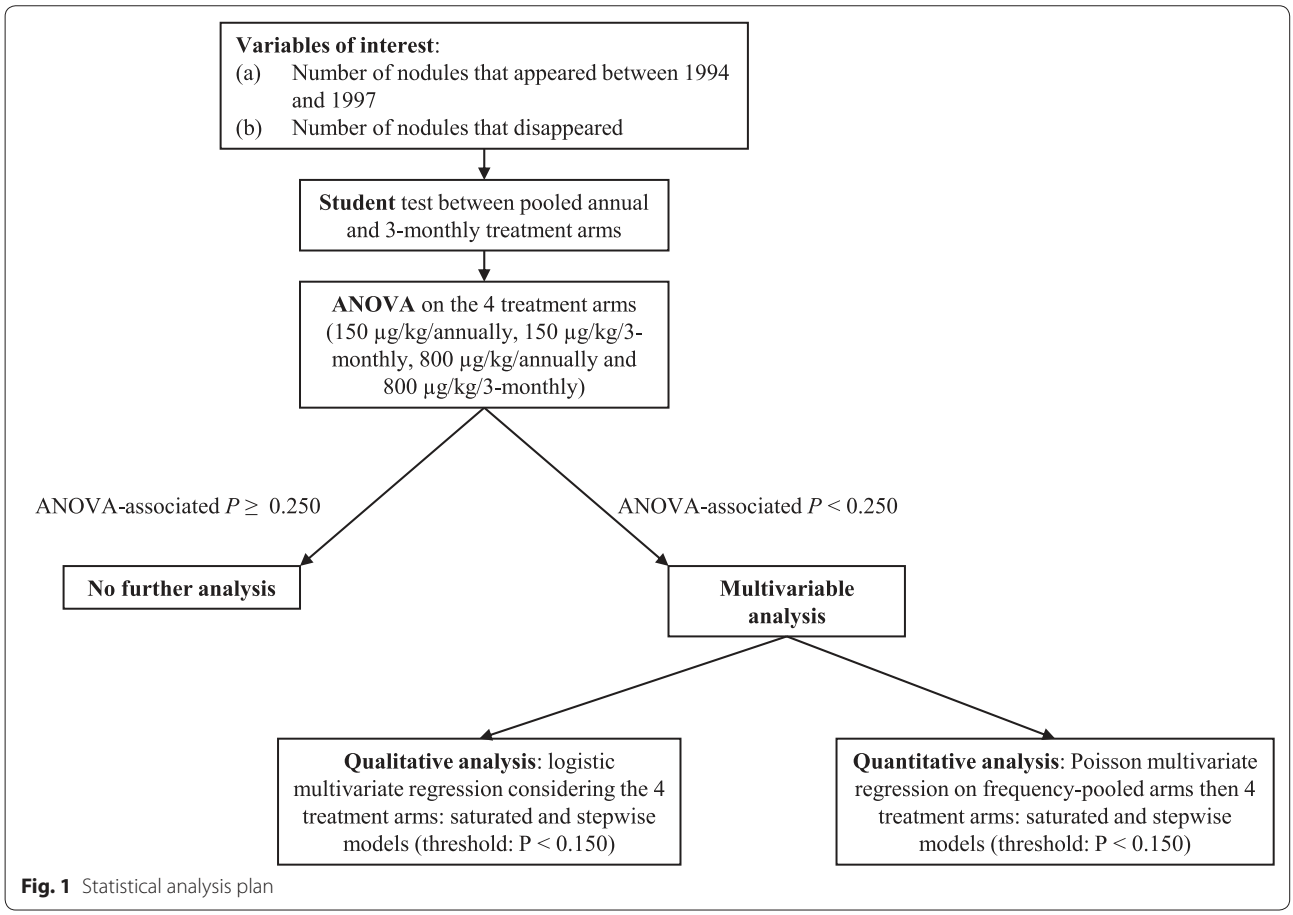


**Fig. 1** Statistical analysis plan

significantly between the treatment groups of subjects with less than 5 palpable nodules. In addition, people with more than 5 nodules and belonging to the groups treated annually with 150 µg/kg and 800 µg/kg had, respectively, 4.1 and 5.5 higher chance to have new nodule(s) than those treated with 150 µg/kg 3-monthly and with less than 5 nodules. The CMFL in the village of residence and the individual MFD were not found to be associated with the probability of appearance of new nodules.

Table 4 shows the results of the Poisson regression model for each frequency of treatment explaining the count of new nodules. It shows that 3-monthly treatment (regression coefficient = 0.026; 95% CI: 0.005–0.048), whatever the dose, is more than twice as effective as annual treatment (regression coefficient = 0.058; 95% CI: 0.035–0.081) to prevent the appearance of nodules. The difference in the slope is significant ($P = 0.001$). Figure 2 represents the predictions of this model. Table 5 shows the results of the Poisson regression model including the four treatment arms separately. For these two regression models, initial individual MFD was associated with the appearance of new nodules. As shown in Figs. 2 and 3, these models reveal a strong interaction between the initial number of nodules and the predicted number of new nodules. It shows that in subjects treated 3-monthly (either with 150 or 800 µg/kg) fewer nodules had appeared than in subjects treated annually (with 150 or 800 µg/kg), and that the difference of appearance was highly correlated with the initial number of nodules harboured by the participants. In addition, treatment with high IVM dose does not seem to influence the number of new nodules, regardless of the number of initial palpable nodules.

### Analysis of disappearance of nodules between 1994 and 1997

In the 248 subjects treated annually, the mean number of nodules which disappeared between 1994 and 1997 (0.46) was lower than in the 237 subjects treated 3-monthly (0.60) but the difference was not significant (t-test: $t_{(483)} = -1.88$, $P = 0.061$). The mean numbers of nodules that disappeared between 1994 and 1997 in subjects of each treatment group are shown in Table 2.

The ANOVA did not show any difference between the four groups (ANOVA: $F_{(3,\ 484)} = 1.29$, $P = 0.279$);

**Table 1** Baseline characteristics of the subjects included in 1994, before the start of the clinical trial

| | All participants | 150 µg/kg annually | 800 µg/kg annually | 150 µg/kg 3-monthly | 800 µg/kg 3-monthly |
|---|---|---|---|---|---|
| No. of subjects | 485 | 126 | 122 | 125 | 112 |
| Mean age ± SD | 36.9 ± 12.0 | 36.4 ± 11.4 | 38.1 ± 12.4 | 36.7 ± 11.9 | 36.6 ± 12.3 |
| Mean weight ± SD (kg) | 63.0 ± 8.0 | 62.7 ± 8.2 | 64.3 ± 7.1 | 62.4 ± 8.5 | 62.7 ± 8.0 |
| Mean no. of nodules in 1994 ± SD | 5.7 ± 2.8 | 5.5 ± 2.5 | 5.8 ± 2.3 | 5.6 ± 3.2 | 5.9 ± 2.9 |
| Median no. of nodules in 1994 (IQR) | 5 (4–7) | 5 (4–7) | 6 (4–7) | 5 (3–7) | 6 (4–7) |
| CMFL in the village of residence: | | | | | |
| Low (*n*, %) | 50 (10.3) | 10 (7.9) | 12 (9.8) | 18 (14.4) | 10 (8.9) |
| Middle (*n*, %) | 135 (27.8) | 39 (31.0) | 37 (30.3) | 30 (24.0) | 29 (25.9) |
| High (*n*, %) | 300 (61.9) | 77 (61.1) | 73 (59.8) | 77 (61.6) | 73 (65.2) |
| Geometric mean of MFD | 56.0 | 64.7 | 50.8 | 48.7 | 61.8 |
| Median of MFD (IQR) | 79 (27–171) | 91 (34–197) | 71 (26–157) | 74 (24–143) | 84 (28–187) |

*Abbreviations*: No, number; SD, standard deviation; IQR, interquartile range; *n*, number of subjects; CMFL, community microfilarial load; MFD, individual microfilarial density (mf/mg)

**Table 2** Appearance and disappearance of the nodules after 3 years of treatment

| | All participants | 150 µg/kg annually | 800 µg/kg annually | 150 µg/kg 3-monthly | 800 µg/kg 3-monthly | *P*-value |
|---|---|---|---|---|---|---|
| No. of subjects | 485 | 126 | 122 | 125 | 112 | |
| Mean no. of new nodules ± SD | 2.1 ± 1.7 | 2.1 ± 1.7 | 2.4 ± 1.8 | 1.7 ± 1.5 | 2.0 ± 1.6 | 0.014 |
| Mean no. of nodules that disappeared ± SD | 0.5 ± 0.8 | 0.5 ± 0.8 | 0.5 ± 0.7 | 0.6 ± 0.8 | 0.6 ± 0.8 | 0.279 |

*Abbreviation*: No, number; SD, standard deviation

**Table 3** Logistic regression of the appearance of nodules

| Variables | Saturated model | | Stepwise model | |
|---|---|---|---|---|
| | OR(95% CI) | *P*-value | OR (95% CI) | *P*-value |
| Appearance of nodule(s) (yes/no) | | | | |
| Less than 5 nodules: | | | | |
| 150 µg/kg 3-monthly (*n* = 66) | Ref. | | Ref. | |
| 150 µg/kg annually (*n* = 77) | 1.6 (0.6–3.8) | 0.32 | 1.6 (0.7–3.8) | 0.28 |
| 800 µg/kg 3-monthly (*n* = 54) | 2.6 (0.8–7.7) | 0.092 | 2.6 (0.9–7.9) | 0.082 |
| 800 µg/kg annually (*n* = 58) | 0.9 (0.4–2.1) | 0.777 | 0.9 (0.4–2.2) | 0.871 |
| More than 5 nodules | | | | |
| 150 µg/kg 3-monthly (*n* = 59) | 0.8 (0.3–1.9) | 0.619 | 0.9 (0.4–2.0) | 0.736 |
| 150 µg/kg annually (*n* = 49) | 3.5 (0.9–13.3) | 0.066 | 4.1 (1.1–15.3) | 0.034 |
| 800 µg/kg 3-monthly (*n* = 58) | 1.2 (0.4–2.9) | 0.759 | 1.3 (0.5–3.2) | 0.577 |
| 800 µg/kg annually (*n* = 64) | 5.2 (1.4–19.5) | 0.014 | 5.5 (1.5–20.1) | 0.01 |
| Age | 1.0 (1.0–1.0) | 0.872 | | |
| Weight | 1.0 (1.0–1.0) | 0.661 | | |
| CMFL in the village of residence | | | | |
| Low | Ref. | | | |
| Middle | 1.2 (0.5–2.9) | 0.704 | | |
| High | 1.3 (0.6–3.0) | 0.537 | | |
| MFD | | | | |
| 0–30 mf/mg | Ref. | | | |
| 31–80 mf/mg | 1.3 (0.6–2.6) | 0.459 | | |
| 81–170 mf/mg | 1.2 (0.6–2.4) | 0.624 | | |
| > 171 mf/mg | 1.7 (0.8–3.7) | 0.151 | | |

Abbreviations: OR, odds-ratio; 95% CI, 95% confidence interval; *n*, number of subjects; CMFL, community microfilarial load; MFD, microfilarial density; Ref., reference

consequently, we did not perform further analysis on the disappearance of nodules.

## Discussion

To the best of our knowledge, our study is the first to compare the effect of various IVM treatment regimens on the appearance of new onchocercal nodules in human subjects exposed to transmission of *O. volvulus*. It demonstrates that the mean number of palpable nodules which appeared within the 3-year period of the trial was significantly lower in the individuals treated 3-monthly with IVM than in those treated annually, and that the high doses had no higher effect than standard doses in reducing the number of new nodules. Strangely, the difference between 800 µg/kg annually and 800 µg/kg 3-monthly was less marked than the difference between 150 µg/kg annually and 150 µg/kg 3-monthly, we have no biological clue to understand this difference, it may be due to unmeasured confounding factors (such as pharmacokinetics or pharmacodynamics characteristics) or to hazards due to serendipity.

We found no significant association between CMFL and appearance of nodules, we hypothesise that with a larger sample size, the association between CMFL exposition and appearance of new nodules may appear.

These results should be interpreted in the light of what is known on the biology of *O. volvulus* within the year following the infective bite. The modalities of development of *O. volvulus* from the initial penetration of the parasite into the host as an L3 to the time when it is found as an adult fecund stage in a nodule are not fully known. The time of the L3-L4 molt has been assessed by *in vitro* studies and by infecting experimentally various animals with L3s of *Onchocerca* spp. The time of the final molt was evaluated by following up the appearance of antibodies, which were assumed to be stage-specific, in a simian model, and by an *in vitro* culturing system. It appears that, for *O. volvulus*, the L3-L4 molt starts 2–3 days after inoculation [11], that the final molt occurs between 1.5 and 2.5 months [12], that the adults become sexually mature at 7.5–11 months, and that the first mf produced by the mature adult female worms can be detected after an average period of 12–15 months [13]. Thus, the lifespan of the immature adult worms would range between five months (7.5 minus 2.5) and 9.5 months (11 minus 1.5). Given this timeframe, one can estimate that

**Table 4** Poisson regression model with pooled arms

| Variable | Saturated model | | Stepwise model | |
|---|---|---|---|---|
| | b (95% CI) | *P*-value | b (95% CI) | *P*-value |
| Increase in no. of new nodules for each additional initial nodule[a] | | | | |
| Annual treatment | 0.055 (0.030–0.080) | < 0.001 | 0.058 (0.035–0.081) | < 0.001 |
| 3-monthly treatment | 0.023 (0.0004–0.046) | 0.045 | 0.026 (0.005–0.048) | 0.014 |
| Age | 0.005 (0.0001–0.010) | 0.045 | 0.006 (0.002–0.009) | 0.002 |
| Weight | − 0.007 (− 0.005–0.003) | 0.703 | | |
| CMFL in the village of residence | | | | |
| Low | Ref. | | | |
| Middle | 0.089 (− 0.153–0.332) | 0.470 | | |
| High | 0.149 (− 0.074–0.372) | 0.191 | | |
| MFD | | | | |
| 0–30 mf/mg | Ref. | | Ref. | |
| 31–80 mf/mg | 0.084 (− 0.108–0.277) | 0.389 | 0.102 (− 0.081–0.285) | 0.275 |
| 81–170 mf/mg | 0.265 (0.082–0.447) | 0.004 | 0.290 (0.117–0.463) | 0.001 |
| > 171 mf/mg | 0.358 (0.181–0.535) | < 0.001 | 0.383 (0.217–0.550) | < 0.001 |
| *P* of the model | < 0.001 | | < 0.001 | |
| AIC | 1648 | | 1644 | |
| BIC | 1686 | | 1669 | |
| Log likelihood | − 815.2 | | − 816.3 | |

[a] The reference is defined as annual treatment and 2 initial nodules

*Abbreviations*: b, regression coefficient; 95% CI, 95% confidence interval; Ref., reference; CMFL, community microfilarial load; MFD, microfilarial density; AIC, Akaike's information criterion; BIC, Bayesian information criterion

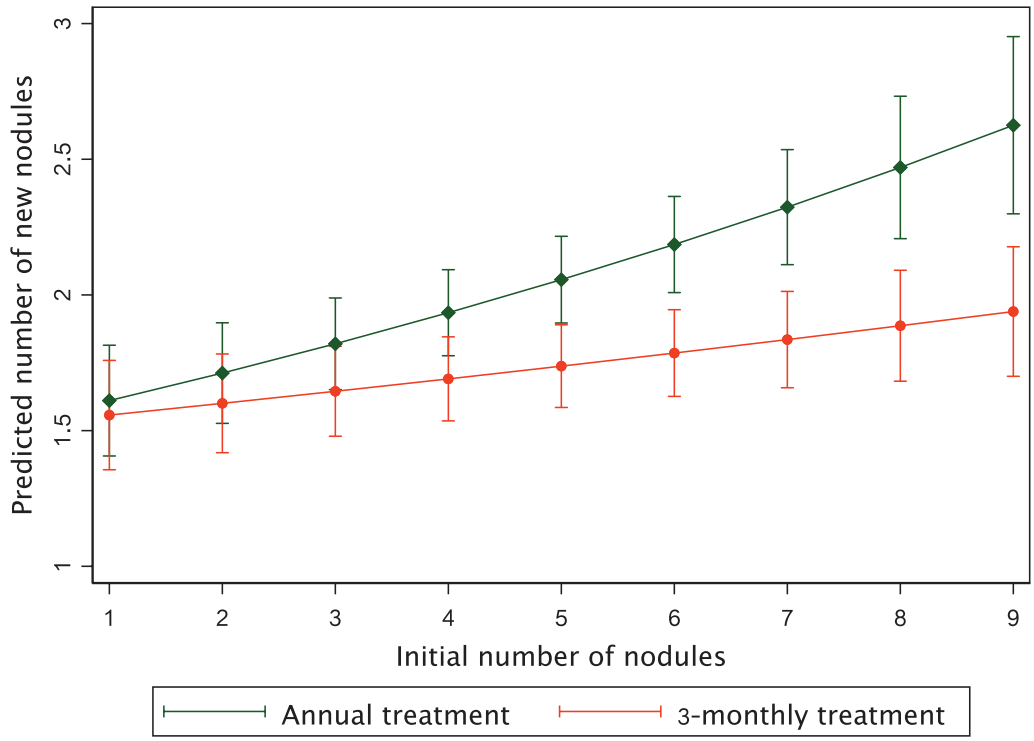


**Fig. 2** Predicted number of new nodules *vs* initial number of nodules (annual *vs* 3-monthly arms). Bars indicate the 95% confidence interval

**Table 5** Poisson regression model (with interaction term between initial number of nodules and treatment arms)

| Variable | Saturated model | | Stepwise model | |
|---|---|---|---|---|
| | b (95% CI) | *P*-value | b (95% CI) | *P*-value |
| Increase in no. of new nodules for each additional initial nodule[a] | | | | |
| 150 µg/kg annually | 0.043 (0.014–0.071) | 0.004 | 0.046 (0.019–0.073) | 0.001 |
| 800 µg/kg annually | 0.067 (0.039–0.095) | < 0.001 | 0.070 (0.043–0.096) | < 0.001 |
| 150 µg/kg 3-monthly | 0.021 (− 0.004–0.047) | 0.106 | 0.024 (− 0.000–0.048) | 0.052 |
| 800 µg/kg 3-monthly | 0.026 (− 0.001–0.053) | 0.063 | 0.029 (0.004–0.056) | 0.026 |
| Age | 0.005 (− 0.00001–0.010) | 0.051 | 0.006 (0.002–0.009) | 0.003 |
| Weight | − 0.001 (− 0.005–0.003) | 0.659 | | |
| CMFL in the village of residence | | | | |
| Low | Ref. | | | |
| Middle | 0.090 (− 0.154–0.333) | 0.470 | | |
| High | 0.151 (− 0.072–0.375) | 0.185 | | |
| MFD | | | | |
| 0–30 mf/mg | Ref. | | Ref. | |
| 31–80 mf/mg | 0.089 (− 0.103–0.281) | 0.364 | 0.105 (− 0.079–0.288) | 0.263 |
| 81–170 mf/mg | 0.278 (0.095–0.461) | 0.003 | 0.301 (0.128–0.474) | 0.001 |
| > 171 mf/mg | 0.371 (0.194–0.549) | < 0.001 | 0.394 (0.227–0.561) | < 0.001 |
| *P* of the likelihood-ratio test for the interaction term: 0.035 | | | | |
| *P* of the model | < 0.001 | | < 0.001 | |
| AIC | 1649 | | 1645 | |
| BIC | 1695 | | 1678 | |
| Log likelihood | − 813.5 | | − 814.6 | |

[a] The reference is defined as 150 µg/kg annually and 2 initial nodules

*Abbreviations*: b, regression coefficient; 95% CI, 95% confidence interval; Ref., reference; CMFL, community microfilarial load; MFD, microfilarial density; AIC, Akaike's information criterion; BIC, Bayesian information criterion

the proportion of L4s exposed to IVM in an individual treated 3-monthly will be between 50% (if the L4s' lifespan is 1.5 months) and 83% (if it is 2.5 months). Regarding immature worms, most would be exposed twice, and a small proportion three times, to the drug. Conversely, given their short lifespan, only a small proportion of L3s "inoculated" to these subjects treated every 90 days would be exposed to the drug. If one assumes that the appearance (or not) of new nodules reflects the effect of the drug on the parasitic stages preceding the mature adult stage, which is debatable (see below), the observed decrease of 17.7% in the number of new nodules in the subjects treated 3-monthly suggests that the prophylactic effect of IVM is not limited to the effects of the drug on the L3s or on the L3-L4 molting process only, but that IVM has also a partial effect on the L4s and/or the immature adult worms. IVM is known to kill L4s and young adults of the canine heartworm *Dirofilaria immitis* and to have a "slow kill" activity against *D. immitis* adults. Indeed, IVM is described as a very effective prophylactic drug against *D. immitis* infection in dogs. Furthermore, we know that the latter parasite is a very close relative to *Onchocerca* spp. Thus, we can hypothesise that IVM works in the same way in *O. volvulus* [14].

In this study, we assumed that the effect of a drug on the L4s and immature adults can be assessed by following up the appearance of new nodules in hosts exposed to transmission of *O. volvulus*. This is debatable because the sites where the L4s and the immature adults live, and the modalities by which the adult female worms are finally found in a nodule, are poorly known. In particular, the extent to which immature females are attracted by existing nodules or are able to create a new nodule are not known. According to Duke et al. [11], "it is expected … that the L4 will be highly mobile and capable (by means unknown) of locating the adult sites of election or (perhaps by means of pheromones) of finding pre-existing worm bundles; and that the immature females may continue these wanderings, …, but are then likely to settle down to form nodules of their own or to join pre-existing nodules. The possibility cannot be excluded that some of the immature females may remain dormant at a prepubertal phase, situated in the connective tissues away from the nodules". Guderian et al. [15] compared the sites of appearance of new nodules in a group of subjects from

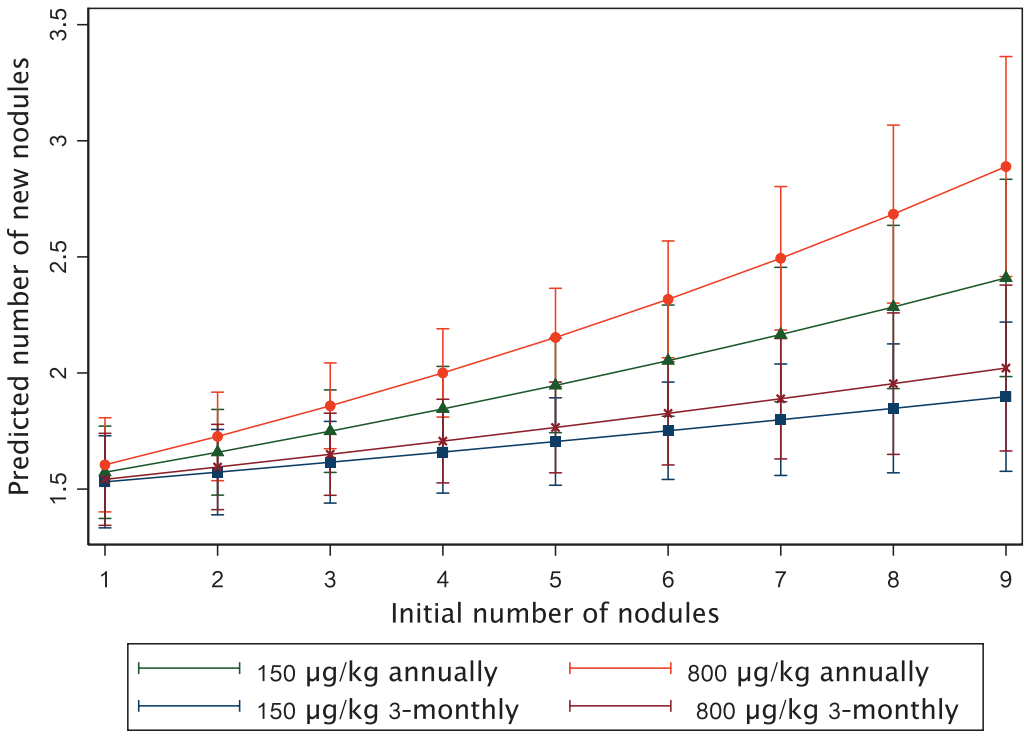


**Fig. 3** Predicted number of new nodules vs initial number of nodules (four treatment arms). Bars indicate the 95% confidence interval

whom all the palpable nodules had been removed and a group with no nodulectomy. They concluded that "It seems likely that young, female, unencapsulated *O. volvulus* are attracted to existing nodules, settle down next to them and then become encapsulated themselves". However, as it is admitted (i) that the female worms, once in a nodule, stay there, and (ii) that nodules form around female worms (not male worms), one may assume that the appearance of a new nodule can occur only if new females have appeared. Therefore, the lower number of new nodules recorded in the groups treated 3-monthly, when compared to the annually-treated group, results probably from an at least partial effect of ivermectin on the L4s and/or the juvenile female worms.

Specific study designs, using probably animal models, could be developed to evaluate the strength of this prophylactic effect after a single dose of IVM, which would help refine the mathematical models used to predict the impact of IVM MDA on onchocerciasis transmission intensity. Trials could also be conducted to define which treatment frequency would be required to obtain the best prophylactic effect. We found that 3-monthly treatments led to a significant reduction in the appearance of new nodules when compared to annual treatment, but the difference was not very marked. Monthly treatments would probably lead to a stronger effect, as suggested by the results of studies conducted on the *O. ochengi*/cattle model [4, 6]. Such monthly treatments have been used in studies evaluating their possible macrofilaricidal effect on *O. volvulus* [16] or their effect on *Loa loa* [17]. They probably cannot be applied on a large scale, but could be proposed to individuals visiting temporarily an onchocerciasis endemic area. Unlike loiasis which can be prevented (totally) using diethylcarbamazine (DEC) [18, 19], no drug is currently proposed to prevent onchocerciasis. Trials using DEC were conducted on chimpanzees experimentally infected with *O. volvulus* and on humans by looking at the effect of the drug on L3s, but the results were not conclusive [20].

A remaining question is why the difference of impact between 3-monthly and annual treatment is higher when the initial number of nodules is higher. Before the start of this study, some participants had more nodules than others. This variability can be explained by different levels of exposure to onchocerciasis transmission, but also by inter-individual heterogeneity in immunological response. Indeed, Tchakouté et al. [21] observed the wide variation in susceptibility between cattle (even when controlling for blackfly exposure). Some individuals are more predisposed than others to tolerate incoming parasites, with a weaker immune response allowing more L3s' and

L4s' developing to the adult stage, and therefore leading to more nodules. During the three years of the trial, it is very unlikely that these two factors changed for the participants. Thus, it makes sense that the most infected people (i.e. with the highest initial number of nodules and highest initial MFD) at the start of the study, are also the most infected ones at the end of the trial.

Few studies tried to evaluate the impact of IVM treatment on nodules' disappearance. Duke et al. [22] assessed this phenomenon by comparing patients who were given IVM at 150 µg/kg 3-monthly and untreated persons. They described a higher proportion of nodules that had disappeared in the treated group but the difference was not significant. Three other studies reported that nodules can disappear after repeated doses of IVM [23–25]. We did not find a difference in the number of nodules that disappeared between the treatment arms of our study, but this could be due to a lack of statistical power due to a small sample size. Further studies have to be conducted to determine the impact of repeated doses of IVM on the nodules' disappearance.

## Conclusions

This study provides evidence that 3-monthly treatment is more effective than annual treatment to prevent the appearance of onchocercal nodules. This effect is particularly marked in individuals with a large number of nodules before treatment. Our results support, for the first time, that ivermectin has probably a prophylactic effect on the L4s and/or the juvenile female *O. volvulus* worms. When the drug is given at a three-month interval, this effect is only partial, but it might be more efficient when given at shorter intervals.

**Abbreviations**

NTD: neglected tropical diseases; WHO: World Health Organization; MDA: mass drug administration; IVM: ivermectin; MFD: microfilarial density; CMFL: community microfilarial load; DEC: diethylcarbamazine.

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**Authors' contributions**

MB designed the study. JC analyzed the data. JC wrote the first manuscript draft. CBC, SDSP and MB reviewed and edited the manuscript. All authors read and approved the final manuscript.

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**Availability of data and materials**

Data supporting the conclusions of this article are included within the article. The datasets used and/or analyzed during the present study are available from the corresponding author upon reasonable request.

**Ethics approval and consent to participate**

This study is the follow-up of a clinical trial approved by the Ethics Committee of Cameroon. The participants in the initial clinical trial all provided written informed consent.

**Consent for publication**

Not applicable.

**Competing interests**

The authors declare that they have no competing interests.

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## Publisher's Note

PLOS NEGLECTED TROPICAL DISEASES

RESEARCH ARTICLE

# Serious adverse reactions associated with ivermectin: A systematic pharmacovigilance study in sub-Saharan Africa and in the rest of the World

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**Data Availability Statement:** Data cannot be shared publicly because of restriction of access to VigiBase. Data are available from the WHO Global

## Abstract

### Background

Ivermectin is known to cause severe encephalopathies in subjects infected with loiasis, an endemic parasite in Sub-Saharan Africa (SSA). In addition, case reports have described ivermectin-related serious adverse drug reactions (sADRs) such as toxidermias, hepatic and renal disorders. The aim of this study was to identify suspected sADRs reported after ivermectin administration in VigiBase, the World Health Organization's global individual case safety reports database and analyze their frequency relative to the frequency of these events after other antinematodal drugs reported in SSA and other areas of the world (ROW).

### Methods

All antinematodal-related sADRs were extracted from VigiBase. Disproportionality analyses were conducted to investigate nervous, cutaneous, psychiatric, respiratory, renal, hepatic and cardiac suspected sADRs reported after ivermectin and benzimidazole drug administration across the world, in SSA and RoW.

### Principal findings

2041 post-ivermectin or post-benzimidazole suspected sADRs were identified including 667 after ivermectin exposure (208 in SSA and 459 in the RoW). We found an increased reporting for toxidermias, encephalopathies, confusional disorders after ivermectin compared to benzimidazole drug administration. Encephalopathies were not only reported from SSA but also from the RoW (adjusted reporting odds ratios [aROR] 6.30, 95% confidence interval: 2.68–14.8), highlighting the fact these types of sADR occur outside loiasis endemic regions.

Individual Case Safety Report (ICSR) database, VigiBase® for researchers who meet the criteria for access to confidential data. Vigibase has the data we used and makes them available to people working in a hospital pharmacovigilance service via the following link: https://vigilyze.who-umc.org/.

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**Competing interests:** The authors have declared that no competing interests exist.

## Conclusion

We described for the first time suspected sADRs associated with ivermectin exposure according to geographical origin. While our results do not put in question ivermectin's excellent safety profile, they show that as for all drugs, appropriate pharmacovigilance for adverse reactions is indicated.

## Author summary

Ivermectin is a drug used worldwide for various indications: onchocerciasis, lymphatic filariasis, strongyloidiasis, human sarcoptic scabies, acarodermatitis and rosacea. In the early 1990s, it was discovered that ivermectin could induce severe encephalopathies in some patients with high parasite loads of *Loa loa*, a filarial nematode. This objective of this pharmacovigilance study is to summarize serious neurological and non-neurological post-ivermectin adverse drug reactions reported in the World Health Organization database called VigiBase. This study shows that reported serious adverse drug reactions associated with ivermectin are fairly consistent with those mentioned in the official product information of ivermectin but also provides some new signals. Serious post-ivermectin encephalopathies can also occur outside of *Loa loa* endemic regions but the understanding of the mechanism by which it occurs requires further studies. A new signal concerning two serious toxidermias (DRESS syndrome and acute generalized exanthematous pustulosis) is also described. A lack of reporting of adverse drug reactions is noticeable in some Sub-Saharan African countries, and actions are needed to increase the reporting rates of these adverse effects in these countries.

## Introduction

Ivermectin is included in the World Health Organization (WHO) list of essential medicines and is commonly used worldwide. Stromectol (ivermectin 3 mg) and its generics (Arrow Lab, Biogaran, Gerda, Mylan, Pierre Fabre, Sandoz, Zentiva) are mainly distributed in Europe and North America. In Europe, ivermectin is labeled for the treatment of strongyloidiasis, diagnosed or suspected infection with *Wuchereria bancrofti* (the filarial nematode causing lymphatic filariasis) or *O. volvulus* (the filarial nematode causing onchocerciasis), and human sarcoptic scabies. In North America, ivermectin is labeled for the treatment of strongyloidiasis and onchocerciasis. Ivermectin is also used off-label in certain cases of acarodermatitis (skin inflammation due to bites of parasitic mites), rosacea and loiasis (the disease caused by the filarial nematode *Loa loa*) [1,2]. In African countries, ivermectin is distributed at single oral doses of 150–200 μg/kg as part of onchocerciasis and lymphatic filariasis elimination programs (the drug, registered under the name of Mectizan for these indications, is donated by Merck & Co., Inc.). It is used as preventive chemotherapy, i.e. distributed annually (sometimes biannually) using a mass drug administration strategy, i.e. to the entire eligible population of the target communities without individual diagnosis.

Ivermectin is a derivative of avermectins. It acts mainly by binding to the glutamate-dependent chloride channels of invertebrate nerve and muscle cells, causing an increase in membrane permeability leading ultimately to neuromuscular paralysis and death of certain parasites. In subjects with high densities of microfilariae (mf, the larval stages of the filarial

parasites) in the skin or the blood, ivermectin is able to induce complex inflammatory reactions called Mazzotti reactions which include pruritus, rash, fever, malaise, lymphadenopathy, arthralgia, tachycardia, hypotension, edema and abdominal pain [3,4]. These reactions reflect the inflammatory phenomena associated with the destruction of mf by the drug. Since the early 1990, ivermectin has been known to cause potentially fatal encephalopathies in individuals with very high microfilarial density of *L. loa* in the blood (loiasis is endemic only in Central Africa) [5,6], also referred to as "Possible/Probable *L. loa* encephalopathy temporally related to Mectizan" (PLERM). PLERM can occur in subjects with *L. loa* microfilarial density >10,000 mf/mL if measured before treatment or >1,000 mf/mL if measured after treatment [7]. Since then, few studies have been conducted to investigate the frequency of these *Loa*-related adverse drug reactions (ADR) and the mechanisms by which they occur [8].

In 2017, an analysis of the WHO Global individual case safety report (ICSR) database (VigiBase) for serious neurological adverse events was conducted [9]. The search identified 52 ivermectin-related ICSRs entered into VigiBase by the pharmacovigilance system of the Democratic Republic of the Congo (DRC) between 2009 and 2013. All patients had central and peripheral nervous system disorders. The mean *L. loa* microfilarial density measured after treatment in these patients was 2149.1 mf/mL, and 61% of them had microfilarial density below 1000 mf/mL, suggesting the possible occurrence of PLERM at low microfilarial density. Another search of the VigiBase was conducted in 2016 to identify serious neurological adverse events other than PLERM after ivermectin administration. The authors found 28 cases of suspected neurological serious ADRs (sADRs) following ivermectin treatment for diseases other than onchocerciasis (10 for scabies, 8 for acarodermatitis, 3 for strongyloidiasis, 5 for lymphatic filariasis, 1 for myiasis and 1 for taeniasis) [10]. This study raised questions about the mechanisms underlying the appearance of these neurological effects. To our knowledge, these studies are the only two that have used a pharmacovigilance database to evaluate the occurrence of post-ivermectin ADRs and they have focused exclusively on neurological events.

Our systematic search of the literature for non-neurological adverse events found 10 cases where ivermectin was associated with cutaneous reactions [11–15], nephropathy [16], psychiatric disorders [17,18], hepatic disorders [19,20] and multiorgan dysfunction syndrome [21]. Clinical trials and observational studies have reported common adverse events such as headache, pruritus, muscle pain, cough, dyspnea, nausea, vomiting, diarrhea, blurred vision, postural hypotension and confusion and more anecdotal effects such as serious skin reactions and edematous swelling [22–24].

In the present study, we searched VigiBase for all the suspected sADRs (not only the neurological ones) reported after ivermectin treatment and after treatment with other antinematodal drugs and conducted disproportionality analyses considering the geographical origin of the reported cases. More specifically, the aims of this study were to identify (i) possible non-neurological pharmacovigilance signals (increased reporting of serious suspected adverse reactions after treatment with ivermectin compared to treatment with other antinematodal drugs), and (ii) possible neurological signals related to indications other than onchocerciasis.

## Methodology

### Data source

Data were extracted from the WHO Global Individual Case Safety Report (ICSR) database, VigiBase [25] which includes more than 20 million cases of suspected ADRs reported by national pharmacovigilance centers in more than 130 countries participating in the WHO Program for International Drug Monitoring [26]. An ICSR is an anonymized report for a single individual who experienced adverse event(s) that may be linked to the use of one or more

drugs. ICSR contains sociodemographic information (age, sex, reporter qualification, country of origin, year of report), information about the drug administration (frequency, dosage, co-medication) and information about the reported adverse event. The latter include the seriousness according to the criteria of the International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use (ICH) [27], adverse event verbatim description and associated terms from the Medical Dictionary for Regulatory Activities (MedDRA) developed by the ICH. All reports of suspected sADRs associated with antinematodal drugs (Anatomical Therapeutic Chemical [ATC] code P02C) from December 2003 (first ever report of ivermectin-associated suspected sADR recorded) up to July 15, 2020 were extracted. Antinematodal drugs included ivermectin, benzimidazole drugs (mebendazole, tiabendazole, albendazole, ciclobendazole, flubendazole, fenbendazole), levamisole, pyrantel, piperazine, diethylcarbamazine, and pyrvinium. Prior to analysis, suspected duplicate reports identified by an automated screening were excluded [28]. When ivermectin had been administered in combination with a benzimidazole or another antinematodal drug, the report was excluded from the analysis. Suspected sADRs were classified following the MedDRA [29], grouped at the System Organ Class (SOC) level and at the individual preferred term (PT) level.

## Study design

We performed disproportionality analyses using the case/non-case method which allows to identify disproportionate reporting, *i.e.* a higher than expected number of adverse reaction reports compared to other reactions recorded in the database by calculating Reporting Odds Ratios (ROR). ROR compares the odds of exposure to ivermectin between cases and non-cases [30,31].

Cases were defined as reports of each suspected sADR of interest identified by a MedDRA PT for severe headache, encephalopathies, confusional disorders, seizures, toxidermias (drug reaction with eosinophilia and systemic symptoms, Stevens-Johnson syndrome, toxic epidermal necrolysis and acute generalized exanthematous pustulosis), psychiatric disorders, suicidal behavior, severe acute respiratory syndrome (SARS), renal disorders, hepatic disorders, cardiac failure, rhythm disorders and Mazzotti reaction. For specific syndromes of interest, we mapped the PTs for the most common symptoms to one variable and used that in the analyses instead of the individual PTs (Table 1).

Non-cases were defined as reports of any other suspected sADR occurring after administration of the same drug.

## Exposure definition

Exposure to ivermectin was identified in the ICSR by the use of ivermectin (ATC code P02CF01) preceding the onset of the serious adverse reaction. Only oral administration of ivermectin was included (topical formulations were excluded).

## Statistical analysis

Descriptive statistics were used to summarize the basic characteristics according to the origin of the ICSR: sub-Saharan Africa (SSA) or the rest of the world (RoW).

Among all suspected sADR reports associated with antinematodal drugs, our primary analyses consisted in calculating the ROR of each suspected sADR of interest (and corresponding 95% confidence interval [95% CI]) for ivermectin compared to benzimidazole drugs using logistic regression models adjusted for age groups, date of the ICSR publication, and origin of the notification (SSA or RoW). The latter can additionally be used as a proxy for ivermectin indication since >99% of subjects with onchocerciasis live in SSA, and ivermectin is usually

**Table 1. Mapping of the PTs for the most common symptoms of syndrome of interest to a new variable.**

| New variable | Algorithm |
|---|---|
| **Encephalopathies** | **At least one of the following PTs** |
| | – Confusion |
| | – Aphasia |
| | – Loss of consciousness |
| | – Coma |
| **Confusional disorders** | **At least one of the following PTs** |
| | – Confusion |
| | – Agitation |
| | – Disorientation |
| **Toxidermias** | **At least one of the following PTs** |
| | – Drug reaction with eosinophilia and systemic symptoms syndrome |
| | – Stevens-Johnson syndrome |
| | – Toxic epidermal necrolysis |
| | – Acute generalized exanthematous pustulosis |
| **Psychiatric disorders** | **At least one of the following PTs** |
| | – Delusion |
| | – Hallucination |
| | – Delirium |
| | – Depersonalization |
| | – Derealization |
| **Renal disorders** | **At least one of the following PTs** |
| | – Renal failure |
| | – Renal impairment |
| | – Renal pain |
| | – Renal injury |
| **Hepatic disorders** | **At least one of the following PTs** |
| | – Hepatitis |
| | – Hepatic failure |
| | – Hepatocellular injury |
| | – Jaundice |
| | – Liver injury |
| | – Hepatic function abnormal |
| **Mazzotti reaction** | **At least two of the following PTs** |
| | – Headache |
| | – Asthenia **or** Fatigue |
| | – Pyrexia **or** Chills |
| | – Arthralgia **or** Myalgia |
| | – Edema **or** Swelling |

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not used for lymphatic filariasis control/elimination outside SSA. To explore a potential effect modification by origin, we performed secondary analyses with stratification according to the origin of the notification (SSA and RoW).

Sensitivity analyses were performed using all antinematodal drugs (including benzimidazoles) as the control group instead of benzimidazoles alone and using the same statistical methods.

For all analyses, the p-values in the Tables are indicated by asterisks: ***: $p<0.01$; **: $p\geq0.01$ to $<0.05$; *: $p\geq0.05$ to $<0.10$. For all analyses, "N/A" means that the category is not available or non-applicable.

Analyses were conducted using STATA v.15.1 software (StatCorps, LP, College Station, TX, USA). Maps were created using the mapCountryData package from R statistical software v. 3.5.0.

## Results

### Descriptive analysis of the sADRs reported after treatment with ivermectin

After elimination of duplicates, 2041 suspected sADRs occurring after administration of antinematodal agents were reported between December 2003 and July 2020, of which 209 (10.2%) resulted in death. A total of 667 suspected sADRs were reported after ivermectin administration: 208 cases in SSA and 459 in the RoW. Table 2 shows the distribution of cases between SSA and RoW by age, gender, who reported the case, brand name, fatality, reporting period, and indication.

Most cases concerned people aged 18–44 years old (43.7%) and were reported by healthcare professionals (90.0%). Mean age (44.7 ± 22.9 years for all cases) was significantly lower for SSA (32.3 ± 14.6 years) than for RoW cases (51.1 ± 23.8 years). Sex distribution was also significantly different between SSA cases (female:male ratio 1:1.96) and RoW cases (1:1). Stromectol, the most frequently brand name reported in the RoW (62.0%), was not reported at all in SSA. Suspected sADRs were more frequently fatal in the RoW (67 deaths; 14.6%) than in SSA (9 deaths; 4.3%). Onchocerciasis was the most frequently reported indication for ivermectin use and this was particularly the case in SSA. Scabies was the second most frequently reported indication for ivermectin use, all cases being from the RoW (96; 28.0%). The reported SOC are presented in Table 3, the three most reported SOC were "General disorders and administration site conditions" (44.4%), "Nervous system disorders" (31.3%) and "Skin and subcutaneous tissue disorders" (30.4%).

The three countries that reported the highest number of cases were the United States of America (152 ICRS, 22.8%), France (151, 22.6%) and the DRC (115, 17.2%). Distributions by country for the 6 most frequently reported SOC (excluding the SOC "Infections and Infestations" and "Injury, poisoning and procedural complications" for which a causal relationship to drug administration is extremely unlikely) are presented across the world in Fig 1 and across Africa and Europe in Figs 2 and 3, respectively.

The most frequently reported suspected sADRs are presented by SOC in S1A, S1B and S1C Table. The ten most frequently reported suspected sADRs of interest are reported in Table 4.

The syndromes of interest which occurred after ivermectin intake and described in Table 1 are reported in Table 5.

Ivermectin indications for the 23 serious encephalopathies which occurred outside SSA were scabies (8), acarodermatitis (4), strongyloidiasis (4), rosacea (1), onchocerciasis (1) and unknown indications (5). Ivermectin indications for the 32 serious encephalopathies which occurred in SSA were onchocerciasis (30), unspecified filariasis (1) and unknown (1). Indications for ivermectin treatment in cases of serious toxidermia were scabies (9), unknown (12), strongyloidiasis (3), lice (3), acarodermatitis (2), cysticercosis (1), onchocerciasis (1), unspecified filariasis (1) and in one case ivermectin had been administered erroneously. Ivermectin indications for cases of serious Mazzotti reactions were onchocerciasis (28), lice (3), parasitosis (1), strongyloidiasis (1), worms (1), filariasis (1) and not reported (7).

**Table 2. Characteristics of sADRs exposed to ivermectin reported in VigiBase according to geographical origin.**

| Characteristics | Sub-Saharan cases (n = 208) | RoW cases (n = 459) | Total (n = 667) |
|---|---|---|---|
| **Age**, n (%) | | | |
| 0–17 | 23 (11.5%) | 32 (8.3%) | 55 (9.4%) |
| 18–44 | 136 (68.3%) | 119 (31.0%) | 255 (43.7%) |
| 45–64 | 36 (18.0%) | 110 (28.6%) | 146 (25.0%) |
| 65–74 | 3 (1.5%) | 45 (11.7%) | 48 (8.2%) |
| >74 | 1 (0.5%) | 78 (20.3%) | 79 (13.5%) |
| Unknown | 9 | 75 | 84 |
| **Gender**, n (%) | | | |
| Male | 137 (66.2%) | 221 (50.0%) | 358 (55.2%) |
| Female | 70 (33.8%) | 221 (50.0%) | 291 (44.8%) |
| Unknown | 1 | 17 | 18 |
| **Reporter type**, n (%) | | | |
| Healthcare professionals | 185 (94.9%) | 380 (87.8%) | 565 (90.0%) |
| Non-healthcare professionals | 10 (5.1%) | 53 (12.2%) | 63 (10.0%) |
| Unknown | 13 | 26 | 39 |
| **Brand name, n (%)** | | | |
| Stromectol | 0 | 285 (62.0%) | 285 (42.7%) |
| Mectizan | 100 (48.1%) | 8 (1.7%) | 108 (16.2%) |
| Others* | 0 | 45 (9.8%) | 45 (6.7%) |
| Unknown | 108 (51.9%) | 122 (26.6%) | 229 (34.3%) |
| **Fatal**, n (%) | | | |
| Yes | 9 (4.3%) | 67 (14.6%) | 76 (11.4%) |
| No | 199 (95.7%) | 392 (85.4%) | 591 (88.6%) |
| **Reporting period**, n (%) | | | |
| ≤ 2012 | 91 (43.7%) | 86 (18.7%) | 177 (26.5%) |
| 2013–2015 | 33 (15.9%) | 144 (31.4%) | 177 (26.5%) |
| 2016–2018 | 70 (33.6%) | 142 (30.9%) | 212 (31.8%) |
| 2019–2020 | 14 (6.7%) | 87 (18.9%) | 101 (15.1%) |
| **Indications**, n (%) | | | |
| Onchocerciasis | 110 (74.8%) | 10 (2.9%) | 120 (24.5%) |
| Scabies | 0 | 96 (28.0%) | 96 (19.8%) |
| Acarodermatitis | 0 | 80 (23.3%) | 80 (16.3%) |
| Strongyloidiasis | 1 (0.7%) | 64 (18.6%) | 65 (13.3%) |
| Filariasis | 29 (19.7%) | 7 (2.0%) | 36 (7.3%) |
| Rosacea | 0 | 27 (7.9%) | 27 (5.5%) |
| Parasitosis | 1 (0.7%) | 20 (5.8%) | 21 (4.3%) |
| Others** | 6 (4.0%) | 39 (11.4%) | 45 (9.2%) |
| Unknown | 61 | 116 | 177 |

* Soolantra (28), Scabioral (7), Sklice (6), Rosiver (2), Driponin (1), Ivermec (1).

** Error (10), Lice (10), Prophylaxis (6), Skin disease (4), Pruritus (3), Cysticercosis (3), Helminth infection (2), Hookworm (2), Schistosomiasis (2), Loiasis (1), Taenia (1), Worms (1).

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## Disproportionality analysis

The results of the disproportionality analyses of sADRs of interest as well as non-cases after administration of ivermectin compared to benzimidazole drugs are presented in Table 6. After

**Table 3. Frequency of reported SOC by regions and in total.** Multiple SOC can be reported in a single ICSR.

| System Organ Class (SOC), n (% of ICSR with mention of the SOC) | Sub-Saharan reports | RoW reports | Total reports |
|---|---|---|---|
| General disorders and administration site conditions | 120 (57.7%) | 176 (38.3%) | 296 (44.4%) |
| Nervous system disorders | 112 (53.8%) | 97 (21.1%) | 209 (31.3%) |
| Skin and subcutaneous tissue disorders | 72 (34.6%) | 131 (28.5%) | 203 (30.4%) |
| Gastrointestinal disorders | 51 (24.5%) | 78 (17.0%) | 129 (19.3%) |
| Infections and infestations | 5 (2.4%) | 77 (16.8%) | 82 (12.3%) |
| Musculoskeletal, connectives tissues disorders | 52 (25.0%) | 26 (5.7%) | 78 (11.7%) |
| Injury, poisoning, procedural complications | 0 | 67 (14.8%) | 67 (10.0%) |
| Psychiatric disorders | 20 (9.6%) | 42 (9.2%) | 62 (9.3%) |
| Respiratory, thoracic, mediastinal disorders | 10 (4.8%) | 52 (11.3%) | 62 (9.3%) |
| Renal and urinal disorders | 28 (13.5%) | 29 (6.3%) | 57 (8.5%) |
| Investigations | 1 (0.5%) | 56 (12.2%) | 57 (8.5%) |
| Eye disorders | 28 (13.5%) | 21 (4.6%) | 49 (7.3%) |
| Hepatobiliary disorders | 1 (0.5%) | 47 (10.2%) | 48 (7.2%) |
| Vascular disorders | 23 (11.1%) | 24 (5.2%) | 47 (7.0%) |
| Blood and lymphatic system disorders | 2 (1.0%) | 42 (9.2%) | 44 (6.6%) |
| Cardiac disorders | 1 (0.5%) | 27 (5.9%) | 28 (4.2%) |
| Metabolism and nutrition disorders | 0 | 24 (5.2%) | 24 (3.6%) |
| Immune system disorders | 2 (1.0%) | 13 (2.8%) | 15 (2.2%) |
| Ear and labyrinth disorders | 6 (2.9%) | 8 (1.7%) | 14 (2.1%) |
| Reproductive system and breast disorders | 6 (2.9%) | 3 (0.7%) | 9 (1.3%) |
| Pregnancy, puerperium, perinatal disorders | 0 | 9 (2.0%) | 9 (1.3%) |
| Neoplasm benign, malignant and unspecified | 0 | 8 (1.7%) | 8 (1.2%) |
| Endocrine disorders | 0 | 7 (1.5%) | 7 (1.0%) |
| Social circumstances | 0 | 4 (0.9%) | 4 (0.6%) |
| Surgical and medical procedures | 0 | 4 (0.9%) | 4 (0.6%) |
| Product issues | 0 | 3 (0.7%) | 3 (0.4%) |
| Congenital, familial and genetic disorders | 0 | 1 (0.2%) | 1 (0.1%) |

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adjustment, the relative frequency of serious headaches reported after treatment with ivermectin and with benzimidazole drugs was similar (adjusted ROR [aROR]: 1.22, 95% CI: 0.83–1.78). This was also the case in origin-stratified analysis (aROR: 1.16, 95% CI: 0.68–1.98 and aROR: 1.39, 95% CI: 0.76–2.53 in SSA and RoW, respectively). In contrast, serious encephalopathies were much more frequently reported after ivermectin than benzimidazole treatment, globally (aROR: 9.23, 95% CI: 4.56–18.61), in SSA countries (aROR: 27.1, 95% CI: 6.34–116.1) and in the RoW (aROR: 6.30, 95% CI: 2.68–14.8). Reports of confusional disorders were strongly associated with ivermectin use globally (aROR: 4.05, 95% CI: 1.81–9.09), in the RoW (aROR: 3.66, 95% CI: 1.49–8.87) but not in SSA (aROR: 3.87, 95% CI: 0.76–19.6). Serious seizures were not more frequently reported after ivermectin than after benzimidazole drugs (aROR: 0.49, 95% CI: 0.49–0.97).

Drug Reaction with Eosinophilia and Systemic Symptoms (DRESS) was more frequently reported with ivermectin than with benzimidazole drugs (aROR: 8.59, 95% CI: 1.85–39.9). No adjustments were possible for the analysis of DRESS because of the low number of cases. Serious toxidermias (DRESS, Stevens-Johnson syndrome, toxic epidermal necrolysis and acute generalized exanthematous pustulosis) were more frequently reported with ivermectin than with benzimidazole drugs globally (aROR: 4.43, 95% CI: 2.07–9.47) and in the RoW (aROR: 6.05, 95% CI: 2.76–13.3), but not in SSA countries (aROR: 0.52, 95% CI: 0.05–5.01). It is

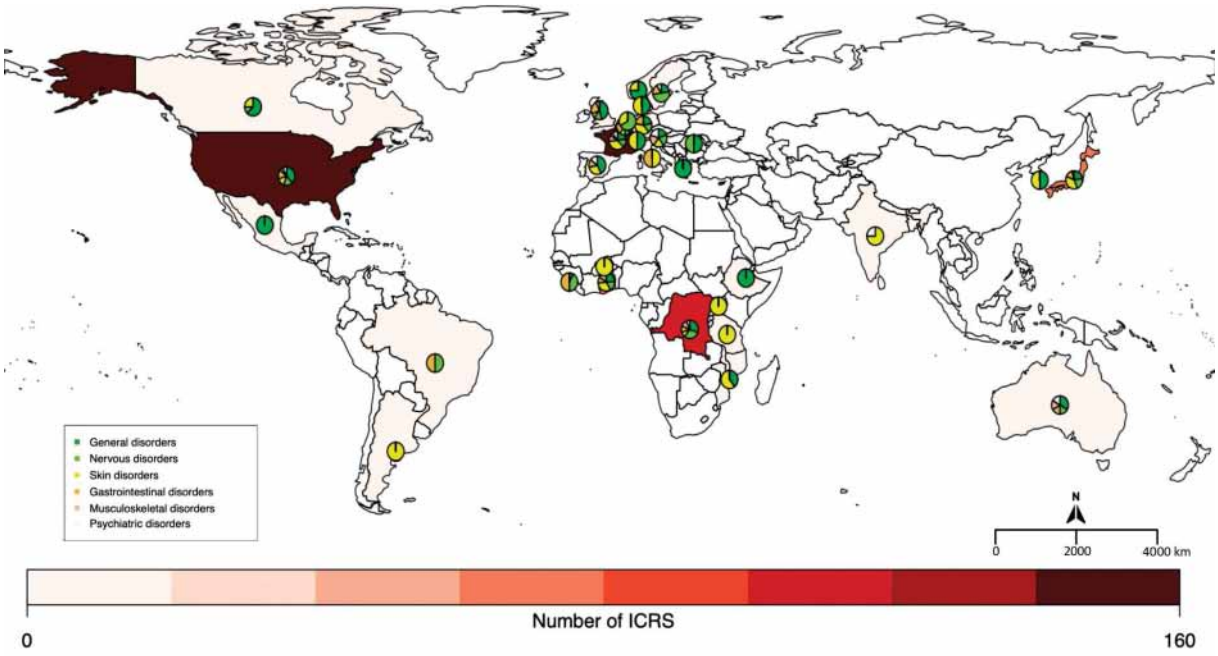


**Fig 1. Number of Individual Case Safety Reports (ICSRs) per country and distribution of sADRs of the 6 most reported SOCs (created with R software and the *Rworldmap* package).** The pie charts show the proportion of the most reported SOCs described by country. The number next to the pie chart represents the number of ICSRs by country (an ICSR can contain multiple SOCs).

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noticeable that eight cases of toxidermia were excluded from the analyses because ivermectin was co-administered with albendazole.

Serious psychotic disorders and suicidal disorders were not more frequently reported with ivermectin than with benzimidazole drugs (aROR: 1.78, 95% CI: 0.70–4.53 and aROR: 7.67, 95% CI: 0.85–69.0, respectively).

Only 5 cases of Severe Acute Respiratory Syndrome (SARS) were reported, and no significant associations were found. aROR values did not indicate any associations for serious hepatic disorders or serious renal disorders either (aROR: 0.51, 95% CI: 0.36–0.74 and aROR: 1.36, 95% CI: 0.66–2.85, respectively).

Serious cardiac failures were significantly associated with ivermectin compared to benzimidazole drug intake (ROR: 11.4, 95% CI: 1.37–94.9, no adjustment possible). Serious rhythm disorders were not found to be associated with ivermectin compared to benzimidazole drugs.

Finally, serious Mazzotti reactions were strongly associated with ivermectin compared to benzimidazole drugs both in SSA (aROR: 1.95, 95% CI: 1.09–3.52) and in the RoW (aROR: 19.7, 95% CI: 2.20–175.5).

## Sensitivity analyses

Disproportionality analyses were repeated with all antinematodal drugs rather than only benzimidazole drugs as control group (S2 Table). Associations for reports of serious headache in RoW (aROR: 1.82, 95% CI: 1.01–3.28) and serious rhythm disorders in RoW (aROR: 3.45, 95% CI: 1.02–11.7) were strengthened in these sensitivity analyses. No changes were found for encephalopathies, confusional disorders, DRESS, toxidermias, seizures, renal disorders, suicidal disorders, psychiatric disorders, SARS, hepatic disorders and Mazzotti reactions. In

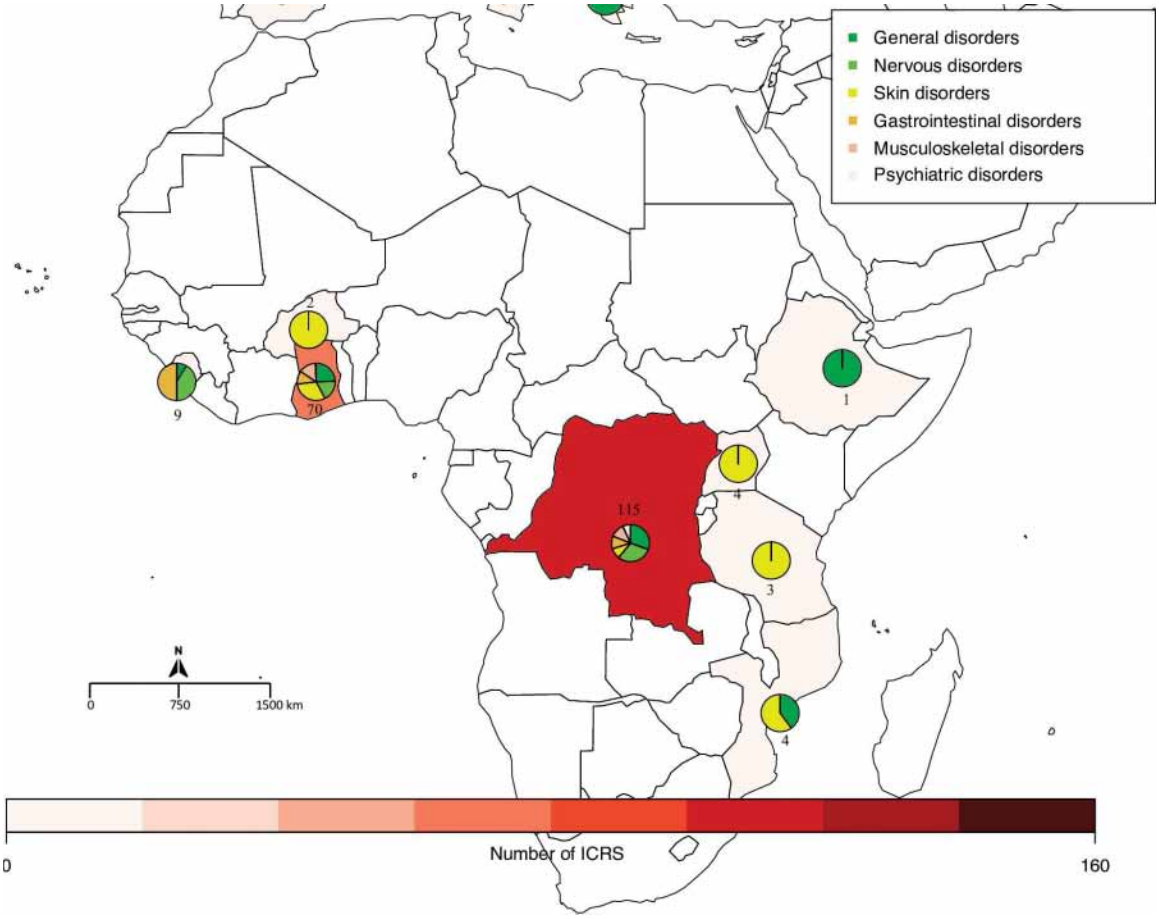


**Fig 2. Number of Individual Case Safety Reports (ICSRs) per African country and distribution of serious ADRs of the 6 most reported SOCs (created with R software and the *Rworldmap* package).** The pie charts show the proportion of the most reported SOCs described by country. The number next to the pie chart represents the number of ICSRs by country (an ICSR can contain multiple SOCs).

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contrast to the primary analysis, the sensitivity analysis identified no association for cardiac failures.

## Discussion

Our study used a case-non-case approach to assess the association between the use of ivermectin and the reporting of neurological as well as non-neurological suspected sADRs, recorded in the WHO drug adverse events database from 2003 to 2020 (see S3 Table for STROBE checklist of case-control studies). To our knowledge, it is the first to globally review the main serious ADRs reported with ivermectin. Some strong significant disproportionality signals were found, showing more frequent reporting of encephalopathies after ivermectin than after benzimidazoles, both in SSA countries and in the RoW. Disproportionality signals were also identified for serious toxidermias, serious confusional disorders and serious Mazzotti reactions with ivermectin when compared with benzimidazole drugs or all non-ivermectin antinematodal drugs. A less consistent signal was found for cardiac failures and further studies are needed to confirm this result.

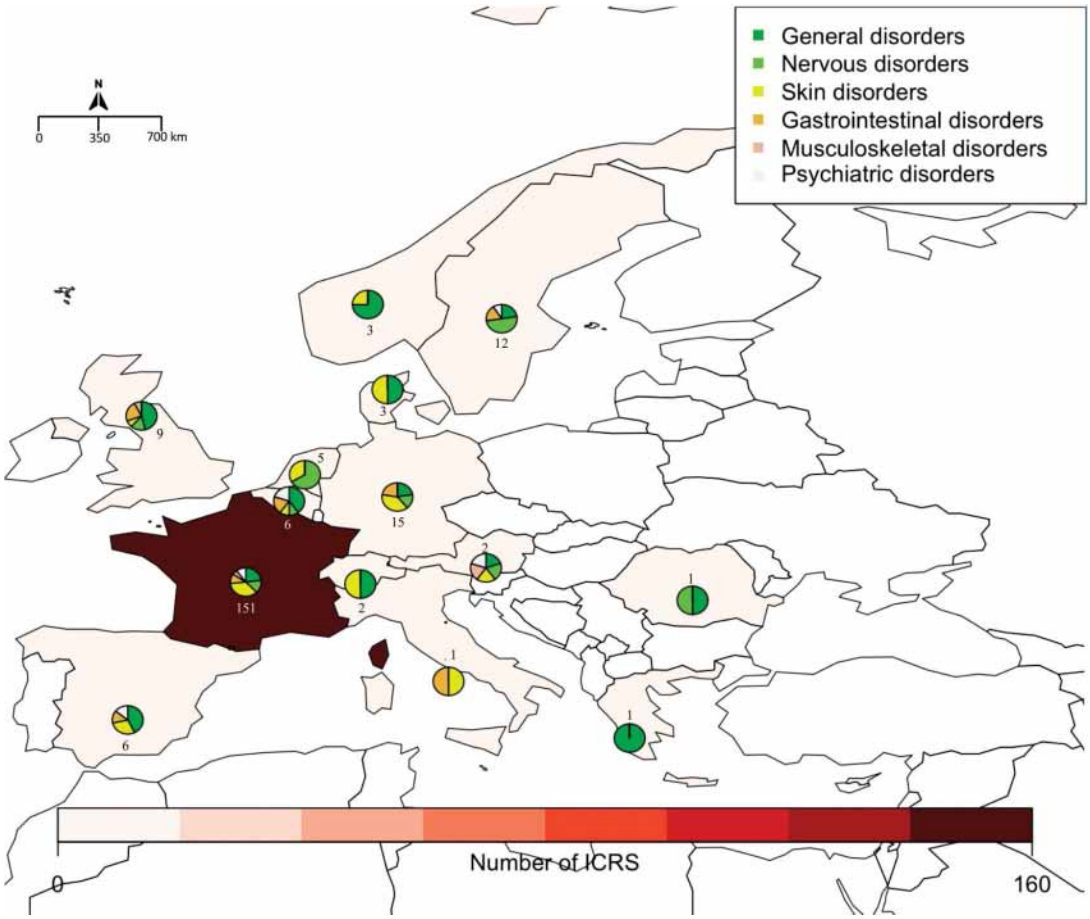


**Fig 3. Number of Individual Case Safety Reports (ICSRs) per European country and distribution of sADRs of the 6 most reported SOCs (created with R software and the *Rworldmap* package).** The pie charts show the proportion of the most reported SOCs described by country. The number next to the pie chart represents the number of ICSRs by country (an ICSR can contain multiple SOCs).

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Some of these results were expected, given the different mechanisms of action of ivermectin and benzimidazoles on the various targeted parasites. Ivermectin exerts a strong microfilaricidal effect on filariae, leading to a destruction of mf within one week after treatment. In subjects

**Table 4. Frequency of ten most reported suspected sADRs by regions and in total.**

| Suspected sADRs, n (%) | Sub-Saharan sADRs | RoW sADRs | Total sADRs |
|---|---|---|---|
| Headaches | 60 (73.2%) | 22 (26.8%) | 82 |
| Asthenia | 61 (78.2%) | 17 (21.8%) | 78 |
| Pruritus | 47 (61.8%) | 29 (38.2%) | 76 |
| Pyrexia | 42 (66.7%) | 21 (33.3%) | 63 |
| Coma | 31 (86.1%) | 5 (13.9%) | 36 |
| Dizziness | 29 (67.4%) | 14 (32.6%) | 43 |
| Vomiting | 16 (50.0%) | 16 (50.0%) | 34 |
| Rash | 9 (29.0%) | 22 (71.0%) | 31 |
| Diarrhea | 20 (66.7%) | 10 (33.3%) | 30 |

https://doi.org/10.1371/journal.pntd.0009354.t004

**Table 5. Frequency of reported suspected syndromes by regions and in total.**

| Suspected syndromes, n (%) | Sub-Saharan cases | RoW cases | Total cases |
|---|---|---|---|
| Encephalopathy | 32 (58.2%) | 23 (41.8%) | 55 |
| Confusional disorders | 6 (27.3%) | 16 (72.7%) | 22 |
| Toxidermia | 8 (24.2%) | 25 (75.8%) | 33 |
| Psychotic disorders | 1 (9.1%) | 10 (90.9%) | 11 |
| Renal disorders | 1 (5.3%) | 18 (94.7%) | 19 |
| Hepatic disorders | 2 (3.8%) | 51 (96.2%) | 53 |
| Mazzotti reaction | 34 (81.0%) | 8 (19.0%) | 42 |

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infected with *Onchocerca volvulus*, the destruction of mf in the skin is associated with inflammatory processes leading to the so-called Mazzotti reaction. In those infected with *L. loa*, the drug probably induces a paralysis of the *L. loa* mf, which are then drained passively in the blood circulation. If the microfilarial density is high, the process can lead to an embolization of mf in the brain capillaries, to inflammatory reactions at the cerebral level, and to an encephalopathy. In contrast, benzimidazoles have little short-term effect on the mf of any filarial species, and thus do not induce Mazzotti reactions, but impair the production of new mf by the adult female worms.

The US Food and Drug Administration (FDA) approved product information for ivermectin mentions that "Rarely, patients with onchocerciasis who are also heavily infected with *Loa loa* may develop a serious or even fatal encephalopathy either spontaneously or following treatment with an effective microfilaricide." [2] In our study, we confirmed the findings of a previous analysis of the data in VigiBase [10] which identified encephalopathies reported with ivermectin also outside of SSA where *L. loa* is not endemic. In addition, we quantified this association by estimating aRORs for ivermectin-induced encephalopathy. aROR was higher in the SSA countries than in the RoW but both were significant, demonstrating a strong global safety signal. Another recent publication described the case of a 13 years old boy presenting a progressive encephalopathy after a single oral dose of ivermectin given at 230 μg per kg, i.e. only slightly higher than the dose used for ivermectin mass drug administration for onchocerciasis (150 μg/kg) and lymphatic filariasis (200 μg/kg) control or to prevent scabies infection (200 μg/kg). The authors found that the patient was a carrier of non-sense mutations in the gene coding for the ATP-binding cassette subfamily B member 1 (ABCB1) transporter which is known to efflux ivermectin from the brain. These mutations can lead to neurological adverse reactions induced by ivermectin [32]. Our results are therefore consistent with the literature and support the evidence of post-ivermectin serious neurological ADRs in some people not infected with *L. loa*. The clinical presentations of *Loa*-related and non-*Loa*-related post-ivermectin neurological ADRs are summarized in Table 7.

The FDA-approved product information for ivermectin also includes the risk of toxic epidermal necrolysis and Stevens-Johnson syndrome as very rare events. By identifying a strong disproportionality signal between ivermectin and benzimidazole drugs or all other antinematodal drugs, our study suggests that ivermectin may be associated with a higher risk of toxidermias than other antinematodal drugs. We also found in VigiBase two types of toxidermia that were never mentioned in the literature: 9 cases of DRESS and 2 cases of acute generalized exanthematous pustulosis after ivermectin intake. These findings could be of great interest for clinicians considering ivermectin treatment in patients at risk for these sADRs or assessing the causality of ivermectin in the development of a toxidermia.

Our study has several strengths. First, we used the global ADRs database VigiBase intended to collect information on suspected ADRs from nearly all national pharmacovigilance centers

**Table 6. Disproportionality analysis of serious adverse reactions associated with ivermectin compared to benzimidazole drugs.**

| Serious adverse drug reaction | Drugs | Cases | Non-cases | Crude ROR (95% CI) | Adjusted ROR [a] (95% CI) | Adjusted ROR [b] in Sub-Saharan Africa (95% CI) | Adjusted ROR [b] in RoW (95% CI) |
|---|---|---|---|---|---|---|---|
| Headache | **Ivermectin** | 71 | 502 | 1.40 ** (1.01–1.93) | 1.22 (0.83–1.78) | 1.16 (0.68–1.98) | 1.39 (0.76–2.53) |
| | Benzimidazoles | 99 | 980 | Ref. | Ref. | Ref. | Ref. |
| Encephalopathy | **Ivermectin** | 55 | 518 | 10.3 *** (5.35–19.9) | 9.23 *** (4.56–18.6) | 27.1 *** (6.34–116.1) | 6.30 *** (2.68–14.8) |
| | Benzimidazoles | 11 | 1068 | Ref. | Ref. | Ref. | Ref. |
| Confusional disorders | **Ivermectin** | 22 | 551 | 3.88 *** (1.87–8.05) | 4.05 *** (1.81–9.09) | 3.87 (0.76–19.6) | 3.66 *** (1.49–8.97) |
| | Benzimidazoles | 11 | 1068 | Ref. | Ref. | Ref. | Ref. |
| Seizure | **Ivermectin** | 11 | 562 | 0.49 (0.25–0.97) | 0.83 (0.40–1.72) | N/A | N/A |
| | Benzimidazoles | 41 | 1038 | Ref. | Ref. | | |
| DRESS * | **Ivermectin** | 9 | 564 | 8.59 *** (1.85–39.9) | N/A | N/A | N/A |
| | Benzimidazoles | 2 | 1072 | Ref. | | | |
| Toxidermia | **Ivermectin** | 25 | 548 | 3.47 *** (1.79–6.73) | 4.43 *** (2.07–9.47) | 0.52 (0.05–5.01) | 6.05*** (2.76–13.3) |
| | Benzimidazoles | 15 | 1065 | Ref. | Ref. | Ref. | Ref. |
| Psychotic disorders | **Ivermectin** | 10 | 563 | 1.72 (0.73–4.08) | 1.78 (0.70–4.53) | N/A | 1.62 (0.62–4.23) |
| | Benzimidazoles | 11 | 1068 | Ref. | Ref. | | Ref. |
| Suicidal behavior | **Ivermectin** | 4 | 569 | 7.58 * (0.84–68.0) | 7.67 * (0.85–69.0) | N/A | N/A |
| | Benzimidazoles | 1 | 1078 | Ref. | Ref. | | |
| SARS ** | **Ivermectin** | 4 | 569 | 7.58 * (0.84–68.0) | N/A | N/A | N/A |
| | Benzimidazoles | 1 | 1078 | Ref. | | | |
| Renal disorders | **Ivermectin** | 17 | 556 | 2.03 ** (1.02–4.05) | 1.36 (0.66–2.85) | N/A | N/A |
| | Benzimidazoles | 16 | 1063 | Ref. | Ref. | | |
| Hepatic disorders | **Ivermectin** | 50 | 523 | 0.61 (0.44–0.86) | 0.51 (0.36–0.74) | 1.36 (0.08–21.9) | 0.50 (0.35–0.73) |
| | Benzimidazoles | 145 | 934 | Ref. | Ref. | Ref. | Ref. |
| Cardiac failure | **Ivermectin** | 6 | 567 | 11.4 ** (1.37–94.9) | N/A | N/A | N/A |
| | Benzimidazoles | 1 | 1078 | Ref. | | | |
| Rhythm disorders | **Ivermectin** | 7 | 566 | 3.32 * (0.97–11.40) | 3.18 * (0.86–11.7) | N/A | 3.18 * (0.86–11.7) |
| | Benzimidazoles | 4 | 1075 | Ref. | Ref. | | Ref. |
| Mazzotti's reaction | **Ivermectin** | 36 | 537 | 2.94 *** (1.74–4.99) | 2.16 ** (1.16–4.03) | 1.95 ** (1.09–3.52) | 19.7 *** (2.20–175.5) |
| | Benzimidazoles | 24 | 1055 | Ref. | Ref. | Ref. | Ref. |

[a] Adjusted for origin (Sub-Saharan Africa or RoW), gender, age and period of notification

[b] Adjusted for gender, age and period of notification

* Drug reaction with eosinophilia and systemic symptoms

** Severe Acute Respiratory Syndrome.

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in the world, allowing us to estimate ROR for rare events with sufficient statistical power and to stratify on geographical origin (SSA vs. RoW). Second, analyses were performed with adjustment for several potential confounders such as origin, gender, age and period of notification. Third, nearly all results of our principal analysis were confirmed in our sensitivity analyses considering all antinematodal agents. Fourth, our results are consistent with already known risk associated with ivermectin (encephalopathy in SSA and Mazzotti reactions).

Limitations of this study include the concern about under-reporting of suspected ADRs and differences in the under-reporting between different countries as well as the lack of information on the number of drug administrations, which is a major disadvantage inherent in studies using pharmacovigilance databases [33,34]. Although under-reporting may be less important since we focused on serious ADRs (which are more likely to be reported) [35], our

**Table 7. Possible/Probable *Loa loa* encephalopathy temporally related to Mectizan and other encephalopathies related to ivermectin: mains risk factors, symptoms and mechanisms involved.**

| | Main risk factors | Main symptoms | Main mechanisms involved |
|---|---|---|---|
| **Possible/Probable *Loa loa* encephalopathy temporally related to Mectizan (PLERM)** | - Intensity of the initial *Loa* microfilaremia | - *12-24h following treatment*: fever, fatigue, arthralgia, agitation, mutism, incontinence<br>- *24-72h following treatment*: consciousness disorders including coma and extrapyramidal signs, typical hemorrhages in the palpebral conjunctiva, retinal lesions<br>- Existence of diffuse pathological process at electroencephalogram level | - Paralysis of the microfilariae due to the action of ivermectin resulting in embolisms in the brain capillaries<br>- Inflammatory processes at the cerebral level |
| **Other encephalopathies related to ivermectin:**<br>**- Toxicosis due to an overdose**<br>**- Toxicosis due to a mutation** | - Polymorphism of MDR1 gene<br>- Deficiency in P-glycoproteins<br>- Intentional or unintentional overdosing | - *Few hours after administration*: nausea, vomiting, abdominal pain, salivation, tachycardia, hypotension, ataxia, pyramidal signs, binocular diplopia<br>- Normal paraclinical tests results | - Passage of ivermectin through the blood-brain barrier (due to overdose or mutation of transporters/metabolism actors) |

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analyses cannot measure the real risk of ADR but only the differences in reported events. Indeed, subjects in the control group (non-cases) are not healthy controls but patients with other various reported ADRs and pharmacovigilance data do not consider the total amount of patients exposed to the drug. Nevertheless, there is no apparent reason that, in a specific region, ADRs would be more or less reported with ivermectin than those occurring after treatment with benzimidazole drugs or other antinematodal drugs. By analyzing real-life surveillance data, disproportionality analyses have demonstrated their usefulness for detecting drug risks [36,37]. Anyway, these results should be taken with caution because of potential missing information. Pharmacovigilance systems are not yet well established in SSA countries. In 2017, only 30% of these countries had specific procedures for the monitoring of ADRs and only 28% had a platform for coordinating pharmacovigilance activities at the national level [38]. Cases of serious adverse events occurring during the ivermectin mass drug administration organized by the onchocerciasis and LF control programs have to be reported by the countries to the Mectizan Donation Program, but the extent to which all relevant observations are recorded in the rural areas where onchocerciasis and LF are endemic and then passed on to the central level is unknown as is the extent to which they are reported into the WHO VigiBase. For example, we found no cases from Cameroon even though ivermectin mass drug administration programs have been ongoing there for 30 years, and many cases are known to have occurred since the early 1990s [39]. In addition, the first case of a post-ivermectin ADR was reported in VigiBase in December 2003 while the first reported death after ivermectin was reported by the WHO Drug Information in 1991 [40]. We consider it likely that availability of complete data from SSA would show more cases associated with ivermectin use and would increase the strength of the safety signals we identified.

In addition, a notoriety bias (selection bias in which a case has a greater chance of being reported if the drug is known to cause, thought to cause, or likely to cause the event of interest [41]) could be considered for reports of encephalopathy in SSA given that the first cases of encephalopathies involving ivermectin led to complications in the early mass drug administration campaigns for elimination of onchocerciasis. However it is unlikely that such bias exist for two reasons (i) in SSA countries, ivermectin is distributed as part of mass treatment organized by the Ministries of Health, and those of *L. loa*-endemic countries might be less inclined to report post-ivermectin sADRs because the cases are not regarded as exceptional and (ii) we also found a strong disproportionality signal in the RoW which is not being affected by this bias.

Our analyses identified serious ADRs that can be associated with ivermectin use that to date have received little, if any attention. As ivermectin is currently widely used off label, especially in Latin America, to control COVID-19 without strong evidence for beneficial effect [42], this study is timely to describe the various suspected sADRs to which this population is potentially exposed even in the absence of onchocerciasis and loiasis endemicity. While ivermectin's excellent safety profile is the basis for mass drug administration campaigns and progress towards elimination in particular of onchocerciasis, one must remain aware and vigilant about the sADRs it may possibly induce.

## Supporting information

**S1 Table. A.** Most frequently reported serious ADRs for each System Organ Class (SOC). If several sADRs belonging to the same SOC are reported in a single patient (ICSR form), the SOC is counted only once in the total. **B.** Most frequently reported serious ADRs for each System Organ Class (SOC) in SSA. If several sADRs belonging to the same SOC are reported in a single patient (ICSR form), the SOC is counted only once in the total. **C.** Most frequently reported serious ADRs for each System Organ Class (SOC) in RoW. If several sADRs belonging to the same SOC are reported in a single patient (ICSR form), the SOC is counted only once in the total.
(DOCX)

**S2 Table. Disproportionality analysis of serious adverse reactions associated with ivermectin compared to other antinematodal drugs.**
(DOCX)

**S3 Table. STROBE Statement—Checklist of items that should be included in reports of case-control studies.**
(DOC)

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ORIGINAL ARTICLE

# Adverse reactions with levamisole vary according to its indications and misuse: A systematic pharmacovigilance study

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Levamisole was initially prescribed for the treatment of intestinal worms. Because of immunomodulatory properties, levamisole has been used in inflammatory pathologies and in cancers in association with 5-fluorouracil. Levamisole is misused as a cocaine adulterant. Post-marketing reports have implicated levamisole in the occurrence of adverse drug reactions (ADRs) and its use is now limited in Europe and North America. In contrast, all other parts of the World continue to use single-dose levamisole as an anthelmintic. The aim of this study was to identify ADRs reported after levamisole exposure in VigiBase, the World Health Organisation's pharmacovigilance database, and analyse their frequency compared to other drugs and according to levamisole type of use.

**Methods:** All levamisole-related ADRs were extracted from VigiBase. Disproportionality analyses were conducted to investigate psychiatric, hepatobiliary, renal, vascular, nervous, blood, skin, cardiac, musculoskeletal and general ADRs associated with levamisole and other drugs exposure. In secondary analyses, we compared the frequency of ADRs between levamisole and mebendazole and between levamisole type of use.

**Results:** Among the 1763 levamisole-related ADRs identified, psychiatric disorders (reporting odds ratio with 95% confidence intervals: 1.4 [1.2–2.6]), hepatobiliary disorders (2.4 [1.9–4.3]), vasculitis (6.5 [4.1–10.6]), encephalopathy (22.5 [17.4–39.9]), neuropathy (4.3 [2.9–7.1]), haematological disorders, mild rashes and musculoskeletal disorders were more frequently reported with levamisole than with other drug. The majority of levamisole-related ADRs occurred when the drug was administrated for a non–anti-infectious indication.

**Conclusion:** The great majority of the levamisole-related ADRs concerned its immunomodulatory indication and multiple-dose regimen. Our results suggest that single-dose treatments for anthelmintic action have a good safety profile.

KEYWORDS
adverse drug reactions, disproportionality, levamisole, pharmacovigilance

## 1 | INTRODUCTION

Levamisole is an old drug derived from imidazothiazole, discovered in 1966 and originally used in veterinary medicine as an anthelmintic and then marketed for the same indication in humans. The anthelminthic action of levamisole is mainly used against *Ascaris lumbricoides*, *Trichuris trichiura* and hookworms (*Necator americanus* and *Ancylostoma duodenale*), the 3 main soil-transmitted helminths (STH) belonging to the World Health Organisation's (WHO's) list of Neglected Tropical Diseases. Because of levamisole's broad spectrum activity and safety, as well as the fact that it is relatively inexpensive and requires only a single oral dose to treat STH, it has been included in the WHO list of essential medicines in 1988.[1] From the early 1980s, national programmes to control STH have typically implemented annual mass drug administration with any of the following anthelmintic drugs: albendazole, mebendazole, levamisole or pyrantel (the exact drug is at the discretion of each country).[2] However, since 2003, the 3 drugs used by national programmes are albendazole, mebendazole and praziquantel (only in combination with 1 of the other 2 treatments), while levamisole is no longer used in mass drug administration according to the WHO data.[3,4] This is due to 3 main reasons: (i) contrarily to levamisole, benzimidazoles (especially mebendazole) are very little absorbed by the organism and remain in the intestine where they kill the intestinal parasites, which is a guarantee of safety; (ii) since 2010, 2 pharmaceutical companies have been donating large quantities of mebendazole (Johnson & Johnson) and albendazole (GlaxoSmithKline) to countries where STH are endemic, thus promoting the use of these molecules from an economic perspective; (iii) albendazole and mebendazole do not require weight adjustment, unlike levamisole, which is used at 2.5 mg/kg. In 2008, Albonico *et al*. reported that "no literature was found specifically on the use of levamisole in pre-school age children" (i.e. as part of mass drug administration for STH infections).[5] However, it appears that the last documented uses of levamisole in national control programmes were in China, Iran, Vietnam, Brazil, Kenya and Nigeria in the 1990s.[5,6]

Besides these national control programmes, levamisole can be purchased with or without a medical prescription for personal use in many countries around the world, particularly in areas where STH are highly endemic (South America, Asia and Africa). For both individual treatment and mass drug administration, levamisole is usually administered as a single oral dose of 2.5 mg/kg or 80 mg for all school-age children to treat STH.[7]

The mechanisms of action of levamisole are multiple and not yet fully elucidated. Levamisole is able to paralyze nematode muscles, leaving the worms unable to attach themselves to the mucous membranes, and causing them to be expelled through the intestine.[8] Levamisole has several other effects on human organisms: it exerts immunomodulatory properties and acts on the dopaminergic, cholinergic and noradrenergic systems.[9] Levamisole was subsequently used for its immunomodulatory action in certain forms of rheumatoid arthritis and in association with 5-fluorouracil in patients with colon cancer or melanoma.[10] In some countries, levamisole is also used in the treatment of paediatric nephrotic syndrome.[11]

### What is already known about this subject

- Levamisole has had many different indications, has been misused and has been associated in the occurrence of serious adverse drug reactions (ADRs).
- This association has led several countries to suspend its use. Nevertheless, other countries still use it daily for its antiparasitic indication and do not report serious ADRs.

### What this study adds

- Most levamisole-related ADRs concern its immunomodulatory proprieties.
- Single-dose treatments of levamisole for an antiparasitic indication appear to have a good safety profile.
- The use of levamisole in specific areas where benzimidazole resistance is feared could be an important resource to overcome the possible occurrence of resistance.

The majority of levamisole-related adverse drug reactions (ADRs) reported when used as an anthelmintic treatment were mild and transient.[12–15] During 1994–2000, some cases of nervous system disorders were reported in North Vietnam but levamisole was produced locally, which, according to the Centre for Adverse Drug Reaction of the Vietnam ministry of health, "raises the issue of quality assurance".[6] In 2009, for the first time, 16 cases of multifocal inflammatory leucoencephalopathy were reported from China after a single dose of levamisole.[16]

The scientific literature also reports various ADRs after levamisole treatment for other purposes than anthelmintic indication. In cancer treatments, levamisole is generally used at high doses (50 mg every 8 h for 3 d) every 2 weeks for at least 1 year[17] and in combination with 5-fluorouracil. Several authors reported cases of multifocal inflammatory leucoencephalopathy,[18–34] vasculitis,[35] agranulocytosis[36] and thrombocytopenia[37] associated with this regimen.

As part of paediatric nephrotic syndrome or rheumatoid polyarthritis, the recommended dose of levamisole is 2 or 2.5 mg/kg on alternate days for 12–24 months.[11] In studies concerning immunomodulatory properties of levamisole, some serious ADRs have been reported: nervous system disorders,[38] vasculitis[39–43] and agranulocytosis.[44,45]

Since 2009, levamisole has been involved in case reports as a cocaine adulterant; the amphetamine-like substance aminorex (an anorectic stimulant) being its metabolite.[46] Several hypotheses have been made to explain cocaine adulteration with levamisole: its cheapness, the large quantity available, its chemical properties, which enable it to go undetected in typically used street purity tests, and/or potentiation of cocaine effects.[47] Severe somatic complications widely reported in users of levamisole-adulterated

cocaine include leucopenia, agranulocytosis, leucoencephalopathy, arthritis, thrombotic vasculopathy and vasculitis.[48] Cardiac complications, cognitive impairments and cerebral toxicities were also recently described.[49–51] As the percentage of levamisole in cocaine powder and the amount of cocaine consumed is never known at the time of consumption, it is very difficult to estimate the level of levamisole exposure in the cases.

Although levamisole is considered as an essential medicine by the WHO, the USAand Europe decided to withdraw its marketing authorization in 2004 and 1998 respectively, and to regulate its use (temporary authorization) for specific indications such as nephrotic syndrome or (as an adjuvant) cancer therapy.

Encephalopathies, vasculitis and agranulocytosis are post-levamisole ADRs whicthath seem related to the type of use of the drug, and thus to the dosage regimen. Besides the adulterated cocaine, information regarding the extent of levamisole use, both in general (including in automedication) and specifically for treatment of STH (i.e. at single dose of 2.5 mg/kg) is scarce. As levamisole has been used in many indications, with very different administration schemes and various coadministered drugs, it is likely that the ADRs occurring varies according to each use. The prescription drug information mentions the following ADRs: neutropenia, thrombocytopenia, leucoencephalopathy, hypersensitive reactions, nervousness, sleepiness, depression, nausea, vomiting, reduced appetite, diarrhoea, constipation, pancreatitis, skin rash and inflammation, muscle and joint pain, inflammation of the mouth, and change of odour.[52] Finally, with the emergence of the COVID-19 pandemic, levamisole has been proposed as a therapeutic strategy option on the basis of its immunomodulatory properties, which were thought to improve clinical status of patients with COVID-19.[53] In this context, we searched the WHO global pharmacovigilance database, VigiBase, for all the suspected ADRs reported after levamisole treatment. We then conducted disproportionality analyses considering the types of use. More specifically, the aims of this study were: (i) to identify new pharmacovigilance signals (increased reporting of suspected ADRs after treatment with levamisole compared to other treatments); (ii) to compare ADRs after levamisole according to its type of use (and therefore its regimen); and (iii) to assess, using all available information, the safety of a single dose. Finally, an overview of the known mechanisms of action of levamisole is provided.

## 2 | METHODS

### 2.1 | Data source

Data were extracted from the WHO Global Individual Case Safety Report (ICSR) database VigiBase,[54] which includes >24 million cases of suspected ADRs reported by national pharmacovigilance centres in >130 countries participating in the WHO Program for International Drug Monitoring.[55] An ICSR is an anonymized report for a single individual who experienced adverse event(s) that may be linked to the use of 1 or more drugs. ICSR contains sociodemographic information (age, sex, reporter qualification, country of origin, year of report), information about the drug administration (frequency, dosage, comedications) and information about the reported adverse event(s). The latter includes the seriousness according to the criteria of the International Council for Harmonization of Technical Requirements for Pharmaceuticals for Human Use (ICH),[56] adverse event verbatim description and associated terms from the Medical Dictionary for Regulatory Activities (MedDRA) developed by the ICH. From VigiBase, all reports of suspected ADRs associated with levamisole from 27 February 1977 (first ever report of levamisole-related suspected ADR recorded) up to 7 February 2021 were extracted. Primary analysis used all reports from VigiBase, comparing levamisole-related ADRs to all ADRs reported in the database (any drugs). Mebendazole-related cases were also extracted and used as control cases because of this drug has similar anthelmintic indications. Prior to analysis, suspected duplicate reports identified by an automated screening were excluded.[57] Suspected ADRs were classified following the MedDRA classification,[58] grouped at the system organ class (SOC) level and at the individual preferred term (PT) level.

### 2.2 | Study design

We performed disproportionality analyses using the case–noncase method, which allows to identify disproportionate reporting, i.e. a higher-than-expected number of adverse reaction reports compared to other reactions recorded in the database by calculating reporting odds ratios (RORs). ROR compares the odds of exposure to levamisole between cases and noncases.[59,60]

Cases were defined as reports of each suspected ADR of interest identified by a MedDRA PT. ADRs of interest were identified from the scientific literature or from the drug official information (summary of product characteristics) and include vasculitis, encephalopathies, peripheral neuropathy, convulsions, agranulocytosis, leucopenia, neutropenia, thrombocytopenia, vertigo, fever, tachycardia, failures (cardiac arrest, cardiorespiratory arrest, heart attack and chest pains), arthritis or synovitis, arthralgia or myalgia and hypothrombinaemia. Because of the small number of PT reported among some SOCs, a global analysis was performed for all PT reported among the 3 following SOCs: "psychiatric disorders", "hepatobiliary disorders" and "renal and urinal disorders". For "skin and subcutaneous tissue disorders" SOC, we conducted 2 separated analyses, 1 on severe skin disorders: Stevens–Johnson syndrome, or toxic epidermal necrolysis or acute generalized exanthematous pustulosis; and 1 on all mild skin disorders (rash or erythema).

Noncases were defined as reports of any other suspected ADR.

### 2.3 | Exposure definition

Exposure was identified in the ICSR by the use of levamisole (Anatomical Therapeutic Chemical [ATC] code P02CE) preceding the onset of the adverse reaction.

### 2.4 | Statistical analysis

Descriptive statistics were used to summarize the basic characteristics according to the indication of levamisole: anti-infectious, immunomodulator, adulterant or unknown/unprecise indication.

The indication categories were retrieved based on the information available in VigiBase and defined as follows. The *anti-infectious* category includes cases where the drug was administered for any infection according to the market authorization, i.e. for parasitic infections, or off-market authorization, i.e. at the discretion of the prescribing physician, for viral or bacterial infections. The *adulterant* category includes all cases where cocaine was part of the coadministered molecules or where the reporter notified that it was a misuse. The *immunomodulator* category includes all cases where levamisole is defined as an adjuvant, immunomodulator or anti-cancer treatment or where 5-fluorouracil was part of the coadministered drugs. Finally, the *unknown/unprecise* category includes all other cases where the reporter did not report any specific indication.

An analysis of characteristics associated with each ADR of interest where levamisole was suspected, including sex ratio, age, percentage of cases considered as serious, median time from initiation of levamisole to effect, reported use, reported period of notification and frequency of administration (single dose or multiple doses), was conducted. The reporting period was categorized into 4 categories (before 1990, between 1990 and 1999, between 2000 and 2009 and after 2009) according to important dates in the history of levamisole (approved in 1970 for its anthelmintic action; approved in 1990 for its immunomodulatory action; loss of marketing authorizations in the 2000s; first case of levamisole uses as a cocaine adulterant in 2009).

Our primary analyses consisted in calculating the ROR of each suspected ADR of interest (and corresponding 95% confidence interval [95% CI]) for levamisole compared to all other drugs reported in VigiBase using logistic regression models. A first secondary stratified analysis consisted in calculating the ROR of each suspected ADR of interest for levamisole compared to mebendazole but only when the indication was anti-infectious. In 2 last secondary analyses, ADRs were compared according to levamisole type of use: immunomodulatory indication *vs*. anti-infectious indication, and adulterant use *vs*. anti-infectious indication.

Analyses were conducted using STATA v.15.1 software (StatCorps, LP, College Station, TX, USA).

### 2.5 | Description of the mechanisms of action of levamisole

A separate literature review was performed using the Medline database to search information on the mechanisms of action of levamisole, their potential implication in occurrence of ADRs and their potential synergy with cocaine or 5-fluorouracil.

## 3 | RESULTS

### 3.1 | Descriptive analysis of the ADRs reported with levamisole

Among the 24 217 750 cases reported in VigiBase and after elimination of duplicates, 1763 suspected ADRs after administration of levamisole were reported between 27 February 1977 and 7 February 2021. Among them, 265 (15.0%) were reported as serious, 89 (5.0%) resulted in death, 82 (4.6%) involved cocaine, 142 (8.0%) occurred after use for an anti-infectious indication, 953 after use for immunomodulatory action (54.1%) and 586 (33.2%) had no specified indication. Within immunomodulatory cases, 51 (5.3%) concerned treatment of paediatric nephrotic syndrome and 902 (94.7%) concerned cancer treatment in association with 5-fluorouracil. Table 1 shows the distribution of cases according to levamisole use by age, sex, dosage regimen, seriousness, reporter type, geographical area and reporting period.

Most cases concerned adults (64.0%) and were reported by healthcare professionals (98.9%). Mean age was 35.7 ± 23.9 years for all cases, 35.7 ± 12.7 years for adulterant cases, 23.4 ± 19.5 years for anti-infectious cases, 59.0 ± 16.7 years for immunomodulator cases, 9.0 ± 3.3 years for nephrotic syndrome and 61.9 ± 11.8 years for cancer therapy. Suspected ADRs were more frequently fatal in adulterant cases (55 deaths; 68.7%) than in other indications. All deaths that occurred in cases where levamisole was used as an adulterant also cited cocaine as a potential suspect. The 4 countries that reported the highest number of cases were the USA (958 cases, 54.2%), the UK (98, 5.5%), France (75, 4.2%) and India (75, 4.2%). Single dose was more frequent in anti-infectious cases than in immunomodulator or unknown indication cases. The 3 most reported SOC were general disorders and administration site conditions (25.8%), nervous system disorders (23.9%) and skin and subcutaneous tissue disorders (20.9%; Table 2).

### 3.2 | Description of ADRs related to levamisole

Description of adverse events where levamisole was suspected are presented in Table 3.

With the exception of convulsions, thrombocytopenia, neuropathy, severe skin disorders and failures, the majority of ADRs were more frequently reported in women than in men. The median time to onset of ADR was lower than 2 weeks for vasculitis, convulsions, thrombocytopenia, severe skin disorders, rashes, vertigo, fever, failures, arthralgia/myalgia and hypothrombinaemia, and higher than 2 weeks for encephalopathy, agranulocytosis, neutropenia, tachycardia and arthritis/synovitis. Relatively few levamisole-related ADRs were reported for the anti-infectious indication and adulterant use, comprising <8% and 5% of reported cases, respectively. Similarly, relatively few ADRs were reported following a single dose of levamisole.

**TABLE 1** Characteristics of levamisole-related adverse drug reactions reported in VigiBase according to indication

| | Levamisole use | | | | |
|---|---|---|---|---|---|
| | Adulterant ($n = 82$) | Anti-infectious ($n = 142$) | Immunomodulator ($n = 953$) | Unknown/Unprecise ($n = 586$) | Total ($n = 1763$) |
| **Age** | | | | | |
| <18 y | 1 (1.4%) | 31 (23.3%) | 49 (6.0%) | 66 (13.4%) | 147 (9.7%) |
| 18–65 y | 69 (97.2%) | 96 (72.2%) | 449 (55.2%) | 353 (71.6%) | 967 (64.0%) |
| >65 y | 1 (1.4%) | 6 (4.5%) | 315 (38.7%) | 74 (15.0%) | 396 (26.2%) |
| Missing data | 11 | 9 | 140 | 93 | 253 |
| **Sex** | | | | | |
| Female | 33 (42.9%) | 72 (51.0%) | 452 (51.9%) | 310 (58.0%) | 867 (53.4%) |
| Male | 44 (57.1%) | 69 (49.0%) | 419 (48.1%) | 224 (42.0%) | 756 (46.6%) |
| Missing data | 5 | 1 | 82 | 52 | 140 |
| **Dosage regimen** | | | | | |
| Single dose | 0 | 72 (60.0%) | 5 (1.5%) | 105 (37.1%) | 182 (25.0%) |
| Multiple doses | 0 | 48 (40.0%) | 319 (98.5%) | 178 (62.9%) | 545 (75.0%) |
| Missing data | 82 | 22 | 629 | 301 | 1036 |
| **Seriousness** | | | | | |
| Yes | 80 (97.6%) | 19 (21.3%) | 42 (70.0%) | 124 (65.3%) | 265 (62.9%) |
| No | 2 (2.4%) | 70 (78.7%) | 18 (30.0%) | 66 (34.7%) | 156 (37.1%) |
| Missing data | 0 | 53 | 893 | 396 | 1342 |
| **Seriousness criterion** | | | | | |
| Caused/prolonged hospitalization | 19 (23.7%) | 5 (38.5%) | 8 (20.0%) | 82 (66.7%) | 114 (44.5%) |
| Death | 55 (68.7%) | 0 | 3 (7.5%) | 31 (25.2%) | 89 (34.8%) |
| Disabling/incapacitating | 0 | 0 | 2 (5.0%) | 0 | 2 (0.8%) |
| Life threatening | 2 (2.5%) | 2 (15.4%) | 1 (2.5%) | 5 (4.1%) | 10 (3.9%) |
| Other important condition | 4 (5.0%) | 6 (46.1%) | 26 (65.0%) | 5 (4.1%) | 41 (16.0%) |
| **Reporter** | | | | | |
| Health professionals | 74 (96.1%) | 108 (99.0%) | 213 (100%) | 433 (98.9%) | 828 (98.9%) |
| Other occupations | 3 (3.9%) | 1 (1.0%) | 0 | 5 (1.1%) | 9 (1.1%) |
| Missing data | 5 | 33 | 740 | 148 | 926 |
| **Continent** | | | | | |
| Africa | 0 | 22 (15.5%) | 0 | 70 (11.9%) | 92 (5.2%) |
| North America | 55 (67.1%) | 1 (0.7%) | 794 (83.3%) | 147 (25.1%) | 997 (56.6%) |
| South America | 0 | 15 (10.6%) | 0 | 56 (9.6%) | 71 (4.0%) |
| Asia | 1 (1.2%) | 79 (55.6%) | 8 (0.8%) | 63 (10.7%) | 151 (8.6%) |
| Australia | 0 | 4 (2.8%) | 40 (4.2%) | 27 (4.6%) | 71 (4.0%) |
| Europa | 26 (31.7%) | 21 (14.8%) | 111 (11.7%) | 223 (38.0%) | 381 (21.6%) |
| **Reporting period** | | | | | |
| Before 1990 | 0 | 15 (10.6%) | 1 (0.1%) | 143 (24.4%) | 159 (9.0%) |
| 1990–1999 | 0 | 10 (7.0%) | 816 (85.6%) | 140 (23.9%) | 966 (54.8%) |
| 2000–2009 | 5 (6.1%) | 26 (18.3%) | 88 (9.2%) | 100 (17.1%) | 219 (12.4%) |
| After 2010 | 77 (93.9%) | 91 (64.1%) | 48 (5.0%) | 203 (34.6%) | 419 (23.8%) |

**TABLE 2** Levamisole-related adverse drug reactions: frequency of reported system organ class (SOC) by indications and in total. Multiple SOCs can be reported in a single Individual Case Safety Report (ICSR)

| SOC, *n* (% of ICSR with mention of the SOC) | Adulterant (*n* = 82) | Anti-infectious (*n* = 142) | Immunomodulator (*n* = 953) | Unknown/Unprecise (*n* = 586) | Total (*n* = 1763) |
|---|---|---|---|---|---|
| General disorders and administration site conditions | 30 (37.0%) | 28 (19.7%) | 221 (23.2%) | 174 (29.7%) | 455 (25.8%) |
| Nervous system disorders | 8 (9.9%) | 36 (25.4%) | 246 (25.8%) | 132 (22.5%) | 422 (23.9%) |
| Skin and subcutaneous tissue disorders | 3 (3.7%) | 30 (21.2%) | 180 (18.9%) | 154 (26.3%) | 369 (20.9%) |
| Gastrointestinal disorders | 3 (3.7%) | 79 (55.6%) | 151 (15.8%) | 118 (20.1%) | 352 (19.9%) |
| Blood and lymphatic system disorders | 13 (16.0%) | 7 (4.9%) | 118 (12.4%) | 96 (16.4%) | 234 (13.3%) |
| Psychiatric disorders | 32 (39.5%) | 14 (9.9%) | 81 (8.5%) | 56 (9.6%) | 183 (10.4%) |
| Musculoskeletal, connectives tissues disorders | 6 (7.4%) | 5 (3.5%) | 98 (10.3%) | 62 (10.6%) | 171 (9.7%) |
| Investigations | 9 (11.1%) | 0 | 92 (9.7%) | 26 (4.4%) | 127 (7.2%) |
| Metabolism and nutrition disorders | 3 (3.7%) | 4 (2.8%) | 99 (10.4%) | 20 (3.4%) | 126 (7.1%) |
| Vascular disorders | 9 (11.1%) | 5 (3.5%) | 45 (4.7%) | 27 (4.6%) | 86 (4.9%) |
| Injury, poisoning, procedural complications | 57 (70.4%) | 0 | 8 (0.8%) | 20 (3.4%) | 85 (4.8%) |
| Respiratory, thoracic, mediastinal disorders | 12 (14.8%) | 4 (2.8%) | 34 (3.6%) | 28 (4.8%) | 78 (4.4%) |
| Infections and infestations | 5 (6.2%) | 1 (0.7%) | 53 (5.6%) | 16 (2.7%) | 75 (4.2%) |
| Hepatobiliary disorders | 0 | 0 | 59 (6.2%) | 13 (2.2%) | 72 (4.1%) |
| Renal and urinal disorders | 5 (6.2%) | 4 (2.8%) | 39 (4.1%) | 17 (2.9%) | 65 (3.7%) |
| Cardiac disorders | 15 (18.5%) | 1 (0.7%) | 26 (2.7%) | 21 (3.6%) | 63 (3.6%) |
| Eye disorders | 0 | 3 (2.1%) | 37 (3.9%) | 18 (3.1%) | 58 (3.3%) |
| Immune system disorders | 0 | 4 (2.8%) | 10 (1.0%) | 8 (1.4%) | 22 (1.2%) |
| Neoplasm benign, malignant and unspecified | 0 | 0 | 15 (1.6%) | 2 (0.3%) | 17 (1.0%) |
| Ear and labyrinth disorders | 0 | 2 (1.4%) | 6 (0.6%) | 8 (1.4%) | 16 (0.9%) |
| Endocrine disorders | 2 (2.5%) | | 9 (0.9%) | 1 (0.2%) | 12 (0.7%) |
| Reproductive system and breast disorders | 0 | 2 (1.4%) | 4 (0.4%) | 2 (0.3%) | 8 (0.5%) |
| Pregnancy, puerperium, perinatal disorders | 1 (1.2%) | 2 (1.4%) | 0 | 1 (0.2%) | 4 (0.2%) |
| Product issues | 2 (2.5%) | 0 | 0 | 1 (0.2%) | 3 (0.2%) |
| Congenital, familial and genetic disorders | 0 | 1 (0.7%) | 1 (0.1%) | 0 | 2 (0.1%) |
| Surgical and medical procedures | 1 (1.2%) | 0 | 0 | 1 (0.2%) | 2 (0.1%) |
| Social circumstances | 1 (1.2%) | 0 | 0 | 0 | 1 (0.1%) |

### 3.3 | Disproportionality analysis

The results of the disproportionality analyses of levamisole-related ADRs of interest compared to any other drugs are presented in Table 4.

The relative frequencies of psychiatric disorders, hepatobiliary disorders, encephalopathies, neuropathy, agranulocytosis, leucopenia, neutropenia, thrombocytopenia, fever, arthritis/synovitis, arthralgia/myalgia, hypothrombinemia and vasculitis were significantly higher with levamisole than with other drugs (see all ROR values and 95% CI in Table 4). Psychiatric disorders were significantly more frequently reported after levamisole-adulterated cocaine intake than after levamisole intake for anti-infectious indication. No cases of hepatobiliary disorders, encephalopathy, neuropathy, agranulocytosis, serious skin disorders (Stevens–Johnson syndrome, or toxic epidermal necrolysis and acute generalized exanthematous pustulosis), arthritis/synovitis, tachycardia, failures or hypothrombinaemia were reported when levamisole was given for an anti-infectious indication. Encephalopathies and leucopenia were more frequently reported when levamisole was used for an immunomodulatory action than when it was used for an anti-infectious indication. Neutropenia was more frequently reported when levamisole was misused than when it was used for its

**TABLE 3** Description of adverse drug reactions related with levamisole

| | | | | Age (*n*, %) | | | Use (*n*, %) | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| **Adverse events** | **Sex Ratio[a]** | **Serious[b]** | **Time of occurrence[c]** | **<18 y** | **18–65 years** | **> 65 years** | **Adulterant** | **Anti-infectious** | **Immunomodulator** | **Unknown indication** |
| **Vasculitis** | 0.31 | 91.7% | 9 | **2** (11.8%) | **14** (82.4%) | **1** (5.9%) | **4** (23.5%) | **1** (5.9%) | **3** (17.5%) | **9** (52.9%) |
| **Encephalopathy** | 0.49 | 100% | 53 | **1** (1.7%) | **32** (54.2%) | **19** (32.2%) | **1** (1.7%) | **1** (1.7%) | **50** (84.8%) | **7** (11.9%) |
| **Neuropathy** | 1.30 | 100% | 47.5 | **0** (0%) | **10** (40.0%) | **7** (28.0%) | **1** (4.0%) | **0** (0%) | **21** (84.0%) | **3** (12.0%) |
| **Convulsions** | 1.37 | 50.0% | 8 | **1** (4.5%) | **10** (45.5%) | **6** (27.3%) | **0** (0%) | **1** (4.5%) | **13** (59.1%) | **8** (36.4%) |
| **Agranulocytosis** | 0.39 | 100% | 38 | **3** (5.0%) | **35** (58.3%) | **14** (23.3%) | **4** (6.7%) | **3** (5.0%) | **18** (30.0%) | **35** (58.3%) |
| **Leucopenia** | 0.72 | 66.7% | 19 | **3** (4.3%) | **43** (61.4%) | **20** (28.6%) | **1** (1.4%) | **1** (1.4%) | **47** (67.1%) | **21** (30.0%) |
| **Neutropenia** | 0.74 | 73.7% | 26 | **16** (28.6%) | **24** (42.9%) | **13** (23.2%) | **7** (12.5%) | **2** (3.6%) | **17** (30.4%) | **30** (53.6%) |
| **Thrombocytopenia** | 1.00 | 75.0% | 9 | **3** (9.4%) | **15** (47.0%) | **11** (34.4%) | **2** (6.3%) | **1** (3.1%) | **22** (68.8%) | **7** (21.9%) |
| **SJS/TEN/APEG[d]** | 1.50 | 100% | 9.5 | **1** (20.0%) | **4** (80.0%) | **0** (0%) | **0** (0%) | **0** (0%) | **3** (60.0%) | **2** (40.0%) |
| **Rashes** | 0.65 | 33.3% | 11 | **21** (11.2%) | **95** (50.8%) | **51** (27.3%) | **0** (0%) | **12** (6.4%) | **109** (58.3%) | **67** (35.6%) |
| **Vertigo** | 0.80 | 58.3% | 1 | **5** (6.7%) | **54** (72.0%) | **11** (14.7%) | **0** (0%) | **16** (21.3%) | **25** (33.3%) | **34** (45.3%) |
| **Fever** | 0.65 | 68.4% | 9 | **11** (8.3%) | **84** (63.4%) | **29** (22.0%) | **5** (3.8%) | **14** (10.6%) | **54** (40.9%) | **59** (44.7%) |
| **Tachycardia** | 0.36 | 100% | 35 | **1** (6.3%) | **13** (81.2%) | **1** (6.3%) | **1** (6.2%) | **0** (0%) | **9** (56.2%) | **6** (37.5%) |
| **Failures** | 1.16 | 95.5% | 2 | **0** (0%) | **34** (80.9%) | **5** (11.9%) | **10** (23.8%) | **0** (0%) | **16** (38.1%) | **16** (38.1%) |
| **Arthritis/synovitis** | 0.75 | MD | 30 | **1** (6.3%) | **7** (43.7%) | **4** (25.0%) | **0** (0%) | **0** (0%) | **12** (66.7%) | **6** (33.3%) |
| **Arthralgia/myalgia** | 0.74 | 86.0% | 2 | **7** (7.0%) | **68** (68.0%) | **15** (15.0%) | **3** (3.0%) | **4** (4.0%) | **57** (57.0%) | **36** (36.0%) |
| **Hypothrombinaemia** | 0.78 | MD | 16.5 | **0** (0%) | **11** (35.5%) | **13** (41.9%) | **0** (0%) | **0** (0%) | **27** (87.1%) | **4** (12.9%) |

MD, missing data.

For age, indication, reporting period and dosage regimen, the lines contain the number of cases and the percentage over all cases. Missing data are not described but are included in the percentage calculations.

[a]Ratio male/female.

[b]Percentage of cases reported as serious.

[c]Median time to onset of effect from initiation of treatment (in days).

[d]Stevens–Johnson syndrome, toxic epidermal necrolysis or acute generalized exanthematous pustulosis.

**TABLE 3** Continued

| | Notification period | | | | Dosage regimen | |
|---|---|---|---|---|---|---|
| Adverse events | Before 1990 | 1990–1999 | 2000–2009 | After 2010 | Single dose | Multiples doses |
| Vasculitis | **2** (12.5%) | **2** (12.5%) | **0** (0.0%) | **12** (75.0%) | **0** (0%) | **5** (29.4%) |
| Encephalopathy | **0** (0.0%) | **42** (71.2%) | **9** (15.2%) | **8** (13.6%) | **0** (0%) | **27** (45.8%) |
| Neuropathy | **2** (8.0%) | **15** (60.0%) | **6** (24.0%) | **2** (8.0%) | **0** (0%) | **8** (32.0%) |
| Convulsions | **1** (4.5%) | **19** (86.4%) | **0** (0%) | **2** (9.1%) | **1** (4.5%) | **12** (54.5%) |
| Agranulocytosis | **21** (35.0%) | **24** (40.0%) | **6** (10.0%) | **9** (15.0%) | **0** (0%) | **28** (53.3%) |
| Leucopenia | **15** (21.4%) | **46** (65.7%) | **6** (8.6%) | **3** (4.3%) | **0** (0%) | **31** (44.3%) |
| Neutropenia | **21** (37.5%) | **5** (8.9%) | **11** (19.6%) | **19** (33.9%) | **0** (0%) | **32** (57.1%) |
| Thrombocytopenia | **5** (15.6%) | **20** (62.5%) | **3** (9.4%) | **4** (12.5%) | **0** (0%) | **14** (43.8%) |
| SJS/TEN/APEG[d] | **0** (0%) | **3** (60.0%) | **0** (0%) | **2** (40.0%) | **0** (0%) | **1** (20.0%) |
| Rashes | **28** (15.0%) | **124** (66.3%) | **14** (7.5%) | **21** (11.2%) | **12** (6.4%) | **67** (35.8%) |
| Vertigo | **3** (4.0%) | **28** (37.3%) | **15** (20.0%) | **29** (38.7%) | **27** (36.0%) | **20** (26.7%) |
| Fever | **34** (25.8%) | **60** (45.5%) | **20** (15.1%) | **18** (13.6%) | **15** (11.4%) | **49** (37.1%) |
| Tachycardia | **2** (12.5%) | **10** (62.5%) | **1** (6.2%) | **3** (18.8%) | **0** (0%) | **5** (31.3%) |
| Failures | **0** (0%) | **17** (40.5%) | **3** (7.1%) | **22** (52.4%) | **9** (21.4%) | **11** (26.2%) |
| Arthritis/synovitis | **1** (6.2%) | **12** (75.0%) | **3** (18.8%) | **0** (0%) | **0** (0%) | **6** (37.5%) |
| Arthralgia/myalgia | **12** (12.0%) | **57** (57.0%) | **11** (11.0%) | **20** (20.0%) | **17** (17.0%) | **25** (25.0%) |
| Hypothrombinaemia | **0** (0%) | **31** (100%) | **0** (0%) | **0** (0%) | **0** (0%) | **6** (19.4%) |

MD, missing data.

For age, indication, reporting period and dosage regimen, the lines contain the number of cases and the percentage over all cases. Missing data are not described but are included in the percentage calculations.

[a]Ratio male/female.

[b]Percentage of cases reported as serious.

[c]Median time to onset of effect from initiation of treatment (in days).

[d]Stevens–Johnson syndrome, toxic epidermal necrolysis or acute generalized exanthematous pustulosis.

**TABLE 4** Disproportionality analysis of levamisole-related adverse reactions at preferred term (PT) level

| | | Primary analysis [a] | | | Secondary analysis 1 [b] | | | Secondary analysis 2 [c] | | | Secondary analysis 3 [d] | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| System organ class | Preferred term | *n* | ROR (95% CI) | *P* | *n* | ROR (95% CI) | *P* | *n* | ROR (95% CI) | *P* | *n* | ROR (95% CI) | *P* |
| Psychiatric disorders | All PT | 183 | 1.4 (1.2–2.6) | .050 | 13 | 4.0 (2.0–7.8) | < .001 | 72 | 0.8 (0.4–1.5) | .509 | 32 | 7.4 (3.5–15.3) | <.001 |
| Hepatobiliary disorders | All PT | 72 | 2.4 (1.9–4.3) | .009 | 0 | No cases* | | 58 | No cases* | | 0 | No cases* | |
| Renal and urinal disorders | All PT | 65 | 1.3 (1.0–2.3) | .065 | 3 | 1.4 (0.4–4.7) | .608 | 42 | 2.1 (0.6–6.9) | .209 | 5 | 3.3 (0.8–14.2) | .108 |
| Vascular disorders | Vasculitis | 17 | 6.5 (4.1–10.6) | <.001 | 1 | 8.3 (0.5–133) | .136 | 3 | 0.4 (0.1–4.3) | .485 | 4 | 7.9 (0.9–72.4) | .066 |
| Nervous system disorders | Encephalopathy | 59 | 22.5 (17.4–39.9) | <.001 | 1 | No cases** | | 50 | 7.8 (1.1–57.0) | .043 | 1 | 1.9 (0.1–30.9) | .650 |
| Nervous system disorders | Neuropathy | 25 | 4.3 (2.9–7.1) | <.001 | 0 | No cases* | | 21 | No cases* | | 1 | No cases* | |
| Nervous system disorders | Convulsions | 22 | 1.4 (0.9–2.4) | .064 | 1 | 0.4 (0.1–3.4) | .444 | 13 | 1.9 (0.2–15.0) | .521 | 0 | No cases*** | |
| Blood and lymphatic system disorders | Agranulocytosis | 60 | 25.2 (19.5–44.7) | <.001 | 3 | No cases** | | 18 | 0.9 (0.2–3.1) | .857 | 4 | 2.6 (0.6–12.0) | .217 |
| Blood and lymphatic system disorders | Leucopenia | 70 | 9.8 (7.7–17.5) | <.001 | 1 | 4.1 (0.4–46.0) | .247 | 47 | 7.3 (1.0–53.5) | .050 | 1 | 1.9 (0.1–30.9) | .650 |
| Blood and lymphatic system disorders | Neutropenia | 56 | 4.8 (3.7–8.5) | <.001 | 2 | 3.3 (0.6–17.3) | .153 | 17 | 1.3 (0.3–5.6) | .749 | 7 | 7.2 (1.4–35.6) | .015 |
| Blood and lymphatic system disorders | Thrombocytopenia | 32 | 2.8 (2.0–4.8) | <.001 | 1 | 4.1 (0.4–46.0) | .247 | 22 | 3.3 (0.4–24.9) | .241 | 2 | 3.9 (0.3–43.3) | .273 |
| Skin and subcutaneous tissue disorders | SJS/TEN/AGEP [e] | 5 | 1.3 (0.6–1.9) | .093 | 0 | No cases* | | 3 | No cases* | | 0 | No cases* | |
| Skin and subcutaneous tissue disorders | All rashes [f] | 187 | 1.5 (1.3–2.8) | .042 | 12 | 0.7 (0.4–1.4) | .337 | 109 | 1.4 (0.7–2.6) | .290 | 0 | No cases*** | |
| General disorders | Vertigo | 75 | 1.1 (0.8–1.9) | .088 | 16 | 1.8 (1.0–3.2) | .038 | 25 | 0.2 (0.1–0.4) | <.001 | 0 | No cases*** | |
| General disorders | Fever | 132 | 2.4 (2.0–4.4) | .008 | 14 | 2.7 (1.5–5.1) | <.001 | 54 | 0.5 (0.3–1.0) | .057 | 5 | 0.65 (0.2–1.9) | .432 |
| Cardiac disorders | Tachycardia | 16 | 1.3 (0.8–2.2) | .074 | 0 | No cases* | | 9 | No cases* | | 1 | No cases* | |
| Cardiac disorders | Failures [g] | 42 | 1.0 (0.7–1.7) | .101 | 0 | No cases* | | 16 | No cases* | | 10 | No cases* | |
| Musculoskeletal disorders | Arthritis/synovitis | 18 | 3.9 (2.4–6.3) | < .001 | 0 | No cases* | | 12 | No cases* | | 0 | No cases* | |
| Musculoskeletal disorders | Arthralgia/myalgia | 100 | 2.3 (1.9–4.2) | .010 | 4 | 4.2 (1.2–14.2) | .020 | 57 | 2.2 (0.8–6.1) | .134 | 3 | 1.4 (0.3–6.6) | .641 |
| Investigations | Hypothrombinaemia | 31 | 41.5 (29.1–70.6) | <.001 | 0 | No cases* | | 27 | No cases* | | 0 | No cases* | |

*n*: number of exposed cases; ROR: reporting odds ratio; CI: confidence interval.

*No cases when levamisole is used as an anti-infective.

**No cases in mebendazole group.

***No cases when levamisole is used as an adulterant.

[a]Primary analysis comparing levamisole-related cases with all cases reported in the World Health Organization pharmacovigilance database.

[b]Secondary analysis comparing adverse drug reactions (ADRs) after use of levamisole for an anti-infective indication with those occurring after use of mebendazole (control group).

[c]Secondary analysis comparing ADRs after use of levamisole for an immunomodulatory indication with those after its use for an anti-infective indication (control group).

[d]Secondary analysis comparing ADRs after use of levamisole for an adulterant action with those after its use for an anti-infective indication (control group).

[e]Stevens–Johnson syndrome, toxic epidermal necrolysis or acute generalized exanthematous pustulosis.

[f]Regroups all rashes and all erythema.

[g]Regroups cardiac arrest, cardiorespiratory arrest, heart attack and chest pains.

anti-infectious action. The association between vasculitis and levamisole intake disappeared in the 3 secondary analyses. Arthralgia/myalgia were also more frequently reported with levamisole compared to mebendazole for anti-infectious purposes. Serious skin and subcutaneous tissue disorders were not more frequently reported with levamisole than with other drugs. Vertigo was more frequently reported with levamisole than with mebendazole and when levamisole was used for an anti-infectious purpose than for an immunomodulatory purpose.

### 3.4 | Levamisole mechanisms of action

Table 5 summarizes the mechanisms of action of levamisole identified from the scientific literature, their potential implication in the occurrence of ADRs and their potential synergy with cocaine or 5-fluorouracil. We identified 11 different pharmacological mechanisms for levamisole. For each identified mechanism, we summarized the pharmacological effects both on worms and humans.

## 4 | DISCUSSION

Our study used a case–noncase approach to analyse data collected in the WHO drug adverse events database from 1977 to 2021 to assess the association between levamisole use and the reporting of suspected ADRs of interest. To our knowledge, it is the first study to review the main ADRs associated with levamisole. Significant disproportionality signals were found, with our results showing more frequent reporting of psychiatric disorders, hepatobiliary disorders,

**TABLE 5** Mechanisms of action of levamisole and their potential synergy of action with 5-fluorouracil and cocaine

| | Potential effects | | | |
|---|---|---|---|---|
| **Mechanisms** | **On worms** | **On humans** | **Potential synergy** | **References** |
| Nicotinic receptor agonist and allosteric modulator | Reduces the capacity of male worms to control their reproductive muscles and limits their ability to copulate | Mimics the effects of acetylcholine on nicotine receptors | Increases the pleasurable and behaviour reinforcing effects of cocaine | 47,61 |
| Inhibition of cyclic AMP-mediated glycogenolysis | Increases glucose incorporation into glycogen and decrease glycogen phosphorylase activity ratios | | | 62 |
| Selective inhibition of MAO-A and COMT | | Resembles certain antidepressant drugs<br>Limits the degradation of dopamine<br>Increases dopamine concentration in the cerebral reward pathway | Potentiates the dopamine level due to cocaine inhibitory action in dopamine reuptake | 63–65 |
| Decrease of norepinephrine reuptake | | Resembles certain antidepressant drugs<br>Convulsions at high doses | Potentiates the norepinephrine release due to cocaine at sympathetic synapsis level | 63,66,67 |
| Anticholinesterase activity | Increases the concentration of acetylcholine | Increases the concentration of acetylcholine | Increases the pleasurable and behaviour reinforcing effects of cocaine | 64,67 |
| Endogenous opioid synthesis | | Increases endogenous opioid concentrations in specific areas of the brain and in peripheral tissues | Potentiates cocaine effects | 65 |
| Metabolization into an amphetamine-like compound (aminorex) | | Modulates norepinephrine, dopamine and serotonin levels | Potentiates cocaine effects | 68–70,78 |
| Local anaesthetic properties | | | | 79 |
| Stimulation of T-cell | | Activates and induces proliferation of T-cells | Potentiates 5-fluorouracil immunomodulatory activities | 9,71 |
| Potentiation of monocyte and macrophage functions | | Increases phagocytosis and chemotaxis | | |
| Increase neutrophil functions | | Increases mobility, adherence and chemotaxis | | |

MAO-A: monoamine oxidase type A; COMT: catechol-omethyl transferase.

vasculitis, encephalopathies, neuropathies, agranulocytosis, leucopenia, neutropenia, thrombocytopenia, mild rashes, fever, arthritis, arthralgia and hypothrombinaemia. When comparing levamisole to mebendazole in anti-infectious indications, we identified new pharmacovigilance signals regarding hepatobiliary disorders, neuropathy, serious skin disorders, tachycardia, failures, arthritis and hypothrombinaemia. In addition, some other known ADR were not retrieved: leucopenia, neutropenia, thrombocytopenia, rashes and hypothrombinaemia. One of our main hypotheses is that levamisole is used at single dose in the vast majority of anti-infectious indications and that this administration regimen results in far fewer ADRs, with this reduction likely to be most significant for serious ADRs. This hypothesis is supported by 2 secondary analyses. Encephalopathies and leucopenia were more frequently reported when levamisole was used for an immunomodulatory action compared to when it was used for an anti-infectious action, and psychiatric disorders and neutropenia were more frequently reported when it was used as an adulterant than for its anti-infectious activity.

The majority of the levamisole-related ADRs concerned either its use in immunomodulatory indications, or when delivered as a multiple-dose regimen. The median times to onset of each ADR suggest that the drug induces short-term effects (vasculitis, convulsions, thrombocytopenia, rashes, vertigo, fever, failures, arthralgia, hypothrombinaemia) as well as delayed effects (encephalopathy, neuropathy, agranulocytosis, leucopenia, neutropenia, tachycardia and arthritis). These delayed effects could be immuno-mediated effects, potentially induced by the immunomodulatory properties of levamisole. If clinical trials on the use of levamisole in patients with COVID-19 give good results, its benefit–risk balance as an immunomodulator in this infection will have to be re-evaluated to enable its use in hospital or ambulatory settings. In June 2021, 4 clinical trials evaluating levamisole in the management of COVID-19 were reported in ClinicalTrials.gov, and the results of 1 of them have been published. The authors conclude that levamisole could potentially improve the cough and dyspnoea of patients with COVID-19 but no benefit could be demonstrated on mortality or aggravation of the disease.[53]

The mechanisms of action of levamisole are multiple. It acts at the level of nicotinic receptors,[47,61] the glucose pathway,[62] dopaminergic pathways,[63–65] norepinephrine and acetylcholine.[63,64,66,67] In addition, secondary mechanisms exist such as its capacity to increase endogenous opiate synthesis,[65] to metabolize into an amphetamine-like compound,[68–70] and to act on the immune system.[9,71] Some of its mechanisms of action are synergistic with those of 5-fluorouracil, used in the treatment of cancers, or with those of cocaine. The fact that levamisole is often administered in combination with cocaine or 5-fluorouracil and the potential synergy of action between these molecules make it difficult to differentiate the molecule most likely to cause certain adverse effects.

Our study has several strengths. First, we used the global ADRs database VigiBase to collect information on suspected ADRs from nearly all national pharmacovigilance systems in the world, allowing us to identify new pharmacovigilance signals for rare events with sufficient statistical power and to stratify on levamisole indication in secondary analyses. Second, our results are consistent with already known risks associated with levamisole (encephalopathy, agranulocytosis and vasculitis). Third, the analysis of real-life surveillance data with disproportionality analyses have already demonstrated their usefulness for detecting drug risks.[72,73]

One of the main limitations of this study, inherent to all studies using pharmacovigilance databases,[74,75] is related to the potential missing information. Under-reporting of suspected ADRs, differences in the capacity of reporting between countries and the lack of information about the total number of patients exposed to the drug may cause biased estimates. Nevertheless, there is no apparent reason why, in a specific region, ADRs would be more or less reported with levamisole than those occurring after treatment with any other drugs. Whilst this might mitigate potential bias in the results presented here, these results should be still interpreted with caution because of this potential missing information. Additionally, pharmacovigilance systems are not yet well established in African countries. In 2017, only 30% of these countries had specific procedures for the monitoring of ADRs and only 28% had a national platform for coordinating pharmacovigilance activities.[76] Despite the widespread usage of levamisole in some African, Latin American or Asian countries, there remains little information about its use or potential ADRs arising from this use. However, our analyses suggest a good safety profile of single-dose levamisole for anthelmintic treatment and its use could be considered in some focal areas where emergence of benzimidazole resistance may occur, due to the high drug pressure caused by mass administration of albendazole or mebendazole.[77]

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## COMPETING INTEREST

The authors claim to have no conflict of interest.

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## DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from VigiBase. Restrictions apply to the availability of these data, which were used under license for this study. Data are available from https://vigilyze.who-umc.org/ with the permission of VigiBase.

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BRIEF REPORT

# A Second Population-Based Cohort Study in Cameroon Confirms the Temporal Relationship Between Onchocerciasis and Epilepsy

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To confirm our earlier evidence of a temporal and dose–response relationship between onchocerciasis and epilepsy, we conducted another cohort study in a different setting in Cameroon. Individuals whose *Onchocerca volvulus* microfilarial density (*Ov*-MFD) was measured in 1992–1994 when they were children were revisited in 2019 to determine if they acquired epilepsy. With reference to individuals with no microfilariae in 1992–1994, the relative risks of acquiring epilepsy were 0.96, 2.76, 3.67, and 11.87 in subjects with initial *Ov*-MFD of 1–7, 8–70, 71–200, and > 200 microfilariae per skin snip, respectively. This study further demonstrates reproducibility using the Bradford Hill's criteria for causality.

**Keywords.** onchocerciasis; epilepsy; causal relationship; Africa; cohort.

Eighty percent of the 50–70 million people with epilepsy (PWE) worldwide are found in low- and middle-income countries [1, 2]. This over-representation of PWE in the general population is particularly patent in Central Africa, where the prevalence is estimated at 59.7 per 1000 people [3]. Furthermore, a meta-analysis showed that the median incidence rate of epilepsy worldwide is 50.4 per 100 000 persons-years, whereas values recorded in Sub-Saharan Africa range between 64 and 187 per 100 000 person-years [3].

A possible association between onchocerciasis and epilepsy was originally suggested in 1938 in Mexico [4]. Several studies and meta-analyses subsequently demonstrated a significant relationship between the 2 diseases [5, 6], even after adjusting for other risk factors and infections [7, 8]. Consequently, the concept of onchocerciasis-associated epilepsy (OAE) was proposed, and in 2015, 381 000 people were estimated to have OAE [9].

A longitudinal study conducted in 2017 in the Mbam valley onchocerciasis focus of Cameroon demonstrated that the incidence of epilepsy was positively correlated with the intensity of *Onchocerca volvulus* infection at a young age (5–10 years old) [10]. This first cohort study provided evidence for 2 of the main Bradford Hill criteria, supporting causality between onchocerciasis and epilepsy (temporality and biological gradient) but had to be replicated in another setting to provide evidence for another criterion, namely consistency (reproducibility). In the present study, we used a design similar to that used in the Mbam valley to evaluate whether the level of infection with *O. volvulus* measured in 1992–1994 in children aged 5–15 years living in the Lékié Division (Center Region, Cameroon) was associated with an increased risk of subsequently developing epilepsy.

## METHODS

### Initial Parasitological Surveys and Selection of Subjects for the 2019 Survey

Between 1992 and 1994, parasitological surveys were conducted in 18 villages of the Lékié Division to measure the levels of *O. volvulus* infection in individuals aged ≥5 years (Table 1; Supplementary Figure 1). Two skin snips were collected from each volunteer using a Holth-type corneoscleral punch and incubated in saline at room temperature for 24 hours. Emerged microfilariae (mf) were counted using a microscope, and the individuals' microfilarial density (MFD), expressed as mf per snip, were calculated using the arithmetic mean of the counts. Community microfilarial load (CMFL), defined as the Williams geometric mean of the MFD in subjects aged ≥20 years, was calculated for each village. In addition, standardized thick blood smears were prepared between 10:00 and 16:00 to measure the participants' *Loa loa* and *Mansonella perstans* MFD (mf/mL). All 18 communities surveyed in 1992–1994 were re-visited in 2019. Since the average age of first seizure in subjects with OAE is between 10 and 14 years old [11, 12], we sought information for all those 1258 individuals who were 5–15 years old during the baseline surveys.

### Evaluation and Definition of Epilepsy

In November 2019, we investigated, with the help of key informants (village authorities, long-standing residents, health workers), whether the selected subjects were still alive and which ones had developed epilepsy. Once identified, we visited, with

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**Table 1. Data by Village**

| Health District | Village | Subjects Targeted | Subjects Retrieved in 2019 | No. (%) SR With *O. volvulus* mf[a] | CMFL[a] | *Loa loa* prevalence[a] | No. (%) of SR with *L. loa* mf[a] | *Mansonella perstans* prevalence[a] | No. (%) SR with *M. perstans*[a] | PY | No. SCE | IR (per 100 000 PY) | 95% CI | CDTI Start Year |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Ebebda | Djouniat | 40 | 37 | 34 (91.9) | 32.4 | 13.1 | 1 (2.7) | 30.3 | 2 (5.4) | 972.2 | 1 | 102.9 | 14.5–730.2 | 1999 |
| Ebebda | Eyene | 85 | 73 | 56 (76.7) | 61.8 | 10.3 | 1 (1.4) | 17.4 | 2 (2.7) | 1981.2 | 0 | 0.0 | N.A. | 1999 |
| Ebebda | Mbenega | 70 | 50 | 47 (94.0) | 53.1 | 4.0 | 1 (2.0) | 8.0 | 3 (6.0) | 1208.7 | 10 | 827.3 | 445.2–1537.6 | 1999 |
| Ebebda | Nega Lendong | 155 | 111 | 90 (81.1) | 29.3 | 21.1 | 8 (7.2) | 10.2 | 5 (4.5) | 2873.6 | 7 | 243.6 | 116.1–511.0 | 1999 |
| Elig Mfomo | Elig Mfomo | 31 | 23 | 11 (47.8) | 2.5 | 31.2 | 4 (17.4) | 32.6 | 2 (8.7) | 573.9 | 2 | 348.5 | 57.2–1393.4 | 1999 |
| Evodoula | Nkolakok | 42 | 35 | 26 (74.3) | 17.6 | 27.3 | 2 (5.7) | 28.1 | 2 (5.7) | 864.0 | 1 | 115.7 | 16.3–821.6 | 1994 |
| Evodoula | Nkolassa | 50 | 26 | 24 (92.3) | 53.8 | 15.7 | 2 (7.7) | 38.6 | 6 (23.1) | 612.3 | 4 | 653.3 | 245.2–1740.7 | 1994 |
| Evodoula | Nkolmeyos I | 35 | 27 | 24 (88.9) | 118.4 | 16.7 | 1 (3.7) | 40.5 | 5 (18.5) | 663.9 | 1 | 150.6 | 21.2–1069.3 | 1994 |
| Monatélé | Nkongmessa | 42 | 23 | 21 (91.3) | 81.3 | 9.6 | 1 (4.4) | 4.1 | 0 | 449.1 | 10 | 2226.5 | 1198.0–4138.1 | 1994 |
| Obala | Nkolfep | 82 | 51 | 4 (7.8) | 0.3 | 31.9 | 7 (13.7) | 3.5 | 0 | 1259.6 | 1 | 79.4 | 11.2–563.6 | 1999 |
| Okola | Ayos | 81 | 73 | 35 (48.0) | 5.7 | 24.9 | 11 (15.1) | 31.4 | 6 (8.2) | 1847.8 | 0 | 0.0 | N.A. | 1999 |
| Sa'a | Lebamzip I | 46 | 27 | 4 (14.8) | 1.3 | 33.1 | 2 (7.4) | 3.1 | 0 | 729.7 | 0 | 0.0 | N.A. | 1999 |
| Sa'a | Nkolbogo I | 70 | 65 | 26 (40.0) | 3.8 | 25.8 | 5 (7.7) | 28.1 | 10 (15.4) | 1735.5 | 0 | 0.0 | N.A. | 1999 |
| Sa'a | Nkolbogo II | 63 | 58 | 38 (65.5) | 22.8 | 26.1 | 8 (13.8) | 54.5 | 29 (50.0) | 1541.5 | 2 | 129.7 | 32.4–518.8 | 1999 |
| Sa'a | Nkolebassimbi | 73 | 53 | 40 (75.5) | 24.8 | 13.4 | 1 (1.9) | 11.4 | 2 (3.8) | 1419.1 | 1 | 70.5 | 9.9–500.3 | 1999 |
| Sa'a | NkoIntsa | 104 | 44 | 13 (29.6) | 2.3 | 28.5 | 6 (13.6) | 8.0 | 0 | 908.0 | 10 | 1101.3 | 592.6–2046.9 | 1999 |
| Sa'a | Nkolossang | 62 | 47 | 10 (21.3) | 3.3 | 26.4 | 5 (10.6) | 18.2 | 3 (6.4) | 1206.0 | 0 | 0.0 | N.A. | 1999 |
| Sa'a | Ntsan Mendouga | 127 | 99 | 52 (52.5) | 10.8 | 18.2 | 6 (6.1) | 13.8 | 3 (3.0) | 2441.3 | 3 | 112.9 | 39.6–381.0 | 1999 |

Abbreviations: CDTI, start year of community-directed treatment with ivermectin; CMFL, *O. volvulus* community microfilarial load (in mf/snip) in the village during the baseline parasitological survey; IR, incidence rate (number of cases per PY); Ov, *Onchocerca volvulus*; PY, person-years; SCE, suspected cases of epilepsy (number); SR, Subjects Retrieved.

[a]Assessed in 1992–1994.

the assistance of local health workers or local authorities, the household of each selected individual or that of their relatives. If the targeted person was not at home, we asked his/her relatives whether he/she was still alive. If the subject had died, the year of death was recorded. For all individuals, a standardized 5-item questionnaire [13] was used to identify "suspected cases of epilepsy" (SCE). SCE was identified when a positive answer was given to at least 1 of the 5 questions. Interviewers had no information on the individuals' MFD measured during the initial parasitological survey. When no information could be obtained from the families, we used the responses from the key informants to define the SCE status. This study was approved by the Cameroon National Ethics Committee for Research in Human Health (registration number 2018/12/1123/CE/CNERSH/SP).

#### Statistical Analyses

The variable of interest was SCE status. Independent variables were gender, age during the initial survey (5, 6–7, 8–9, 10–11 [reference group], 12–13, or 14–15 years), MFD (0 [reference group], 1–7, 8–70, 71–200, and > 200 mf/snip), the presence of blood *L. loa* mf (negative vs positive), the presence of *M. perstans* mf, CMFL measured in 1992–1994 in the subject's village of residence (<4, 4–19, 20–29, and ≥30 mf/snip), and the start year of community-directed treatment with ivermectin (CDTI) for onchocerciasis control in the health district (HD; 1994 or 1999). HDs are health administrative units in charge of the implementation of health programs.

Data (individual duration of follow-up) concerning individuals who were not identified as SCE and who died between the initial survey and 2019 were censored by the year of death if it was known, or at half-time of the follow-up period if it was not known. Data concerning the SCE were censored at half-time of the follow-up period if the patient was alive or at half-time of the period between baseline and the year of death if declared dead. Incidence rates (IRs) were estimated by dividing the number of SCE by the total number of person-years of follow-up. Then, in order to assess individual risk factors associated with SCE (incidence rate ratios [IRRs]), we performed a multivariate Poisson regression model including all the independent variables mentioned above.

All possible and relevant interactions as well as random effects on the HD were assessed using likelihood ratio tests. All analyses were performed with Stata (version 14.0).

## RESULTS

#### Population Interviewed and Incidence Rates

In 2019, information on SCE could be obtained for 922 of the 1258 targeted subjects (73.3%). The mean follow-up period for these 922 subjects was 25.2 years, and the number of person-years of follow-up was 23 287. Fifty-three SCE were identified, including 45 through questionnaires applied directly to the person or his/her relatives, and 8 were identified through questionnaires applied to key informants. The overall IR of epilepsy was 53/23 287 (227.6 per 100 000 person-years). The IR increased gradually with the initial MFD, with values ranging from 117.8 per 100 000 persons-years for individuals without skin mf to 952.8 per 100 000 persons-years for those with an MFD >200 mf/snip ($P < .0001$) (Table 2).

#### Individual Risk Factors Associated With SCE

The risk of being identified as an SCE in 2019 was higher for those who were 5 years old during the initial surveys than for the 10-year-olds (adjusted IRR [aIRR], 3.60; $P < .0001$). There was no difference between genders or between CMFL categories (after adjustment for individual MFD). The risk increased gradually with the individual MFD: aIRRs for individuals with 1–7, 8–70, 71–200, and >200 mf/snip were 0.96 ($P = .937$), 2.76 ($P = .017$), 3.67 ($P < .001$), and 11.87 ($P < .001$), respectively. No significant association was found between SCE status and presence of *L. loa* or *M. perstans* microfilaremia, or start year of CDTI. No interactions between the covariates were found. Inclusion of a random effect at the HD level ($P = .090$) did not affect the strength and significance of the effect of the MFD on SCE status.

## DISCUSSION

This study confirms the results obtained in the neighbouring Mbam division [10]: a temporal relationship between onchocerciasis and epilepsy and a dose–effect relationship with a risk of developing epilepsy increasing gradually with the MFD during childhood. This study also confirms the absence of any gender effect but an increased risk for the younger children, all other parameters being equal. Lowering the minimal age of inclusion in CDTI (presently 5 years) should be considered.

Considering that all villages in a given HD benefitted from similar CDTI-related activities, the random effect was evaluated at the HD level (not the village level). As this random effect was close to significance, the risk of developing epilepsy could vary slightly between the HDs.

Unexpectedly, the start year of CDTI was not associated with SCE incidence. However, one should consider that the HDs where CDTI started in 1994 had the highest onchocerciasis endemicity levels. Although there is a possible lack of statistical power, one may consider that the first years of CDTI in these HDs had little impact on the intensity of transmission and/or that children were less treated than adults during the first years of CDTI implementation. As individual history of treatment is lacking, it is impossible to support one hypothesis or the other.

As in the previous study [10], our current study presents these possible biases: (i) the possible misclassification of SCE for individuals with a history of provoked seizures, (ii) the absence of control for other possible risk factors (eg, cysticercosis), (iii)

**Table 2. Population Study, Follow-up Data, Incidence Rates, and Incidence Rate Ratios**

| | | No. Examined in Baseline Study (% of Total) | No. With Information Collected in 2017 (% of Total) | SCE, No. | PY | IR (per 100 000 PY) (95% CI) | *P*[a] | Model Without Random-Effect | | Model With a Random-Effect at HD Level | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | | aIRR[b] (95% CI) | *P* | aIRR[c] (95% CI) | *P* |
| Total | | 1258 | 922 | 53 | 23 287.4 | 227.6 (173.9–297.9) | | | | | |
| Age, y | 5 | 95 (7.6) | 59 (6.4) | 7 | 1445.8 | 484.1 (230.8–1015.6) | .380 | 3.60 (2.19–5.92) | <.0001 | 3.39 (1.25–9.14) | .016 |
| | 6–7 | 273 (21.7) | 182 (19.7) | 6 | 4673.4 | 128.4 (57.7–285.8) | | 0.83 (0.37–1.85) | .645 | 0.74 (0.26–2.09) | .572 |
| | 8–9 | 222 (17.7) | 169 (18.3) | 8 | 4285.5 | 186.7 (93.4–373.3) | | 1.03 (0.35–3.31) | .956 | 1.07 (0.42–2.74) | .889 |
| | 10–11 | 273 (21.7) | 212 (23.0) | 10 | 5410.4 | 184.8 (99.4–343.5) | | Ref | | | |
| | 12–13 | 235 (18.7) | 182 (19.7) | 12 | 4556.2 | 263.4 (149.6–463.8) | | 1.06 (0.54–2.07) | .868 | 0.99 (0.42–2.36) | .991 |
| | 14–15 | 160 (12.7) | 118 (12.8) | 10 | 2916.0 | 342.9 (184.5–637.4) | | 1.19 (0.436–3.86) | .775 | 1.17 (0.47–2.90) | .735 |
| Sex | Female | 637 (50.6) | 449 (48.7) | 25 | 11 216.6 | 222.9 (150.6–329.9) | .892 | Ref | | Ref | |
| | Male | 621 (49.4) | 473 (51.3) | 28 | 12 070.8 | 232.0 (160.2–336.0) | | 0.75 (0.33–1.73) | .505 | 0.78 (0.44–1.38) | .388 |
| CMFL, mf/snip | <4 | 395 (31.4) | 257 (27.9) | 13 | 6412.7 | 202.7 (117.7–349.1) | .004 | Ref | | Ref | |
| | 4–19 | 250 (19.9) | 207 (22.4) | 4 | 5153.1 | 77.6 (29.1–206.8) | | 0.24 (0.04–1.36) | .106 | 0.29 (0.08–1.02) | .054 |
| | 20–29 | 291 (23.1) | 222 (24.1) | 10 | 5834.1 | 171.4 (92.2–318.6) | | 0.32 (0.07–1.47) | .143 | 0.20 (0.10–0.93) | .036 |
| | ≥30 | 322 (25.6) | 236 (25.6) | 26 | 5887.5 | 441.6 (300.7–648.6) | | 0.51 (0.09–2.86) | .447 | 0.49 (0.15–1.66) | .251 |
| Ov in skin snip | Negative | 524 (41.7) | 367 (39.8) | 11 | 9341.8 | 117.8 (65.2–212.6) | .004 | | | | |
| | Positive | 734 (58.43) | 555 (60.2) | 42 | 13 945.6 | 301.2 (222.6–407.5) | | | | | |
| MFD, mf/snip | 0 | 524 (41.7) | 367 (39.8) | 11 | 9341.8 | 117.8 (65.2–212.6) | <.001 | Ref | | Ref | |
| | 1–7 | 241 (19.2) | 182 (19.7) | 4 | 4638.1 | 86.2 (32.4–229.8) | | 0.96 (0.38–2.43) | .937 | 0.93 (0.28–3.07) | .909 |
| | 8–70 | 245 (19.5) | 178 (19.3) | 10 | 4558.8 | 219.4 (118.0–407.7) | | 2.76 (1.20–6.35) | .017 | 2.66 (0.98–7.19) | .054 |
| | 71–200 | 129 (10.3) | 100 (10.9) | 7 | 2544.8 | 275.1 (131.1–577.0) | | 3.67 (1.86–7.21) | <.001 | 3.29 (1.01–10.71) | .048 |
| | >200 | 119 (9.5) | 95 (10.3) | 21 | 2203.9 | 952.8 (621.3–1461.4) | | 11.87 (5.56–25.33) | <.001 | 11.60 (3.89–34.61) | <.0001 |
| *Loa loa*, mf/mL | Negative | 1077 (85.6) | 796 (86.3) | 47 | 20 046.7 | 234.5 (176.2–312.0) | .484 | Ref | | Ref | |
| | Positive | 99 (7.9) | 72 (7.8) | 2 | 1848.7 | 108.2 (27.1–432.6) | | 0.46 (0.14–1.55) | .211 | 0.46 (0.11–1.96) | .292 |
| | Missing | 82 (6.5) | 54 (5.9) | 4 | 1391.9 | 287.4 (107.9–765.7) | | 1.46 (0.75–2.83) | .265 | 1.52 (0.48–4.77) | .476 |
| *Mansonella perstans*, mf/mL | Negative | 1074 (85.4) | 788 (85.5) | 43 | 19 857.7 | 216.5 (160.6–292.0) | .679 | Ref | | Ref | |
| | Positive | 102 (8.1) | 80 (8.7) | 6 | 2037.7 | 294.4 (132.3–655.4) | | 0.93 (0.31–2.75) | .896 | 1.15 (0.45–2.97) | .770 |
| | Missing | 82 (6.5) | 54 (5.9) | 4 | 1391.9 | 287.4 (107.9–765.7) | | N.A. | | N.A. | |
| CDTI | 1994 | 169 (13.4) | 111 (12.0) | 16 | 5589.4 | 617.9 (378.6–1008.6) | <.001 | Ref | | Ref | |
| | 1999 | 1089 (86.6) | 811 (98.0) | 37 | 20 698.0 | 178.8 (129.5–246.7) | | 0.63 (0.11–3.80) | .617 | 0.49 (0.13–1.75) | .270 |

Abbreviations: aIRR, adjusted incidence rate ratio; CDTI, start year of community-directed treatment with ivermectin; CI, confidence interval; CMFL, community microfilarial load (in mf/snip); HD, health district; IR, incidence rate (number of cases per PY); MFD, individual microfilarial density (in mf/snip); Ov, *Onchocerca volvulus* mf; PY, persons-years; SCE, suspected cases of epilepsy (number).

[a]*P* values were calculated within each variable and assessed using the log-rank test for sex, skin snip positivity, *Loa loa* microfilariae positivity, *Mansonella perstans* positivity, CDTI, and the trends modified log-rank test for age, CMFL, and skin snip in 5 categories of variables.

[b]Multivariate logistic model with a cluster-robust standard errors to account for possible intra-community clustering.

[c]Multivariate logistic model with a random effect at the HD level.

and the absence of confirmation of epilepsy by detailed neurological examination of the SCE.

Nevertheless, this study provides significant new findings, investigating for the first time the possible role of *L. loa* and *M. perstans* in inducing epilepsy and demonstrating that it was not the case.

In conclusion, this study supports the previous findings and consequently adds the reproducibility principle to the Bradford Hill's criteria [14], supporting the causal nature of the relationship between *O. volvulus* infection and epilepsy.

## Supplementary Data

Supplementary materials are available at *Open Forum Infectious Diseases* online. Consisting of data provided by the authors to benefit the reader, the posted materials are not copyedited and are the sole responsibility of the authors, so questions or comments should be addressed to the corresponding author.

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# Effect of Lymphatic Filariasis and Hookworm Infection on Pregnancy Course and Outcome in Women Living in the Democratic Republic of the Congo

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*Abstract.* Little is known about the effect of helminth infections on the natural gynecological and pregnancy course. Our goal was to assess the relationship between *Wuchereria bancrofti* and hookworm (HW) infections with pregnancy course and outcome in a group of 82 women living in a rural area of the Democratic Republic of the Congo. Demographics and information on gynecological and obstetrical histories were collected retrospectively with standardized questionnaires. *Wuchereria bancrofti* and HW infections were diagnosed using a filarial antigen-detection test and the Kato–Katz method, respectively. Analyses consisted of multivariable logistic regressions adjusting for age, number of deliveries, and history of anthelmintic treatment (HAHT). The median age of study participants was 35 (interquartile range [IQR]: 30–44) years, and the median number of deliveries was five (IQR: 3–7). *Wuchereria bancrofti* and HW infection rates were 44.5% and 43.3%, respectively. Filarial antigenemia and HW infection were not significantly associated with the number of deliveries. The proportions of women with a history of pregnancy resulting in neonatal death, miscarriage, premature birth, and postpartum hemorrhage were 56%, 44%, 23%, and 36%, respectively. History of pregnancy associated with neonatal death was less frequent in women with HAHT, tended to be more frequent in women with filarial antigenemia, and was not associated with HW infection. None of the three other pregnancy events studied (miscarriage, premature birth, and postpartum hemorrhage) were associated with filarial antigenemia or HW infection. The positive association found between HAHT and lower risk of neonatal death warrants investigation in larger groups of women.

## INTRODUCTION

Many infectious diseases can cause infertility in males or females, as well as lead to adverse pregnancy outcomes.[1–3] Infertility, defined as "a disease of the reproductive system defined by the failure to achieve a clinical pregnancy after 12 months or more of regular unprotected sexual intercourse,"[4] can arise because of "male factors" (such as alterations in sperm concentration and/or motility and/or morphology), female factors, or both. The most common causes of female infertility are ovulatory disorders, tubal occlusion or abnormalities, pelvic adhesions, and endometriosis. Sexually transmitted infections with bacteria *Chlamydia trachomatis* and *Neisseria gonorrhoeae* are a major cause of endometritis and of salpingitis, both of which can result in tubal occlusion and pelvic adhesions, leading to infertility.[5] Protozoan infections have also been reported as causes of male and female infertility due to local inflammatory processes or hormonal disorders arising from infection.[6] Evidence also supports potential impacts of helminthic parasites on fertility: infection with *Schistosoma haematobium* can cause tubal occlusion, leading to infertility and ectopic pregnancy,[7–10] and infection with *Schistosoma* sp. can also induce hormonal imbalances and dysregulation associated with infertility.[10] Infertility was found to be significantly associated with residence in areas of high *S. haematobium* prevalence in East Africa.[11] Adult stages of the filarial worm *Wuchereria bancrofti* (the main cause of lymphatic filariasis [LF]) have been found in nodules or lymphatics of the genital tract, where they have been shown to lead to salpingitis, blockage of the fallopian tubes, and ectopic pregnancy.[12–15] Microfilariae of *W. bancrofti* and other filarial species such as *Loa loa* and *Mansonella perstans* have been found in the follicular fluid and in cervicovaginal smears, but the impact of this presence on fertility is uncertain.[16–19] Results of a single community-wide study suggest that LF infection has no effect on fertility, despite a strong positive association being found between being microfilaremic and abnormal menstruation patterns in women aged ≥ 30 years.[20] It has also been suggested that *L. loa* and *M. perstans* microfilaremia may affect the functions of the hormonal system, leading to delays in puberty, disturbances to the menstrual cycle, and infertility in older subjects.[21] Soil-transmitted helminths (STH) might also have an impact on fertility. In a longitudinal study conducted in Bolivia, infection with *Ascaris lumbricoides* was found to be associated with earlier first births and shortened inter-birth intervals, whereas infection with hookworm (HW) was associated with delayed first pregnancy and extended inter-birth intervals.[22] Whether this impact of STH on fertility is due to immunological phenomena is a matter of debate.[23] The worms *Enterobius vermicularis* and, to a lesser extent, *A. lumbricoides* can induce tubo-ovarian lesions, leading to infertility or ectopic pregnancies,[24,25] but these cases are exceptional, and STH do not seem to have a significant impact on the reproductive potential before the implantation of the embryo in the uterus.

Viral, bacterial, and parasitic infections can also impact the pregnancy outcomes, by increasing the risk of abortion, congenital anomalies, stillbirth, intrauterine growth retardation, preterm birth, or neonatal death.[26–31] Besides the ectopic pregnancies described earlier, the effects of maternal infection with *Schistosoma* sp. on the pregnancy outcomes are not well known.[32–35] The same uncertainties exist regarding the effect of maternal filarial infections. In some studies, risk of abortion and/or postnatal deaths was higher in females with filariasis than that in controls, or higher in areas

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where LF was highly endemic than in non-endemic areas.[36,37] Evidence is mixed however; in a study conducted in Sri Lanka, neither gestation age at delivery nor birthweight differed significantly between females with or without circulating filarial antigens (CFAs).[38] The effects of maternal infection with *Schistosoma* sp. or STH on the pregnancy outcomes have been assessed by several cross-sectional or longitudinal studies, including clinical trials with anthelmintic drugs. Because of extensive variability in study designs, outcomes assessed, and levels of infection across studies, as well as numerous potential confounding factors, the effects of *Schistosoma* and STH on pregnancy outcomes remain unclear.[39–42] In this context, we conducted a retrospective study in two villages in the Democratic Republic of the Congo (DRC) to evaluate the effect of LF and HW (*Necator americanus* or *Ancylostoma duodenale*) on pregnancy outcomes in female residents.

## METHODS

**Study population and selection of subjects.** The study was conducted in June–July 2018 in two neighboring villages (Misai and Mbumkimi) located in the Kwilu Province of the DRC. These villages had been previously selected for a community trial which started in 2014 and whose objective was to assess the impact of community biannual treatment with albendazole alone on LF and STH infections.[43,44] During this trial, all individuals aged ≥ 2 years were offered treatment with a single dose of albendazole (400 mg) every 6 months, and volunteers aged ≥ 5 years had yearly parasitological assessment for LF and STH infections. For the purpose of the present study, we invited women aged at least 15 years in 2018 to answer a standardized questionnaire including sociodemographic and obstetric history information. This questionnaire was applied by the nurse in charge of these two villages. Consenting women were included only if they had been married for at least 2 years.

**Obstetric history and outcomes.** The following information was collected from surveyed women: number of deliveries, number of living children, number of dead children, number of miscarriages, number of children born before 8 months of pregnancy (preterm birth), number of newborns who died within 1 month of delivery (neonatal mortality), and number of postpartum hemorrhages (see questionnaire in Supplementary Material). Statistical analyses focused on four pregnancy adverse outcomes derived from these surveys: history of miscarriage, history of preterm birth, history of neonatal mortality, and history of postpartum hemorrhage, all represented as binary (presence/absence) variables. We also assessed the relationship between the number of deliveries and the infection (either LF or HW) status of the participants.

**Parasitological status regarding LF and STH.** From 2014, all participants in the albendazole trial were invited to undergo a rapid test assessing their LF infection status. This test was delivered before their first treatment and then annually thereafter. This was performed using the Filarial Test Strip (FTS) which detects CFAs in the blood (indicative of the presence of live adult worms).[45] The test was used according to the manufacturer's instructions (Alere, Scarborough, ME). Most participants in the present study had received their first albendazole treatment in 2014 and were followed up until the date of the present study in 2018. The first FTS result obtained during the trial was used in the analyses (as a proxy for chronic LF infection) to define LF-infected versus LF-non-infected women. The use of a recent result (at the time of inclusion in the albendazole trial) as a proxy for the historical level of infection in the participants is relevant because longitudinal studies (up to 26 years of follow-up) have shown that, in the absence of treatment, individual status regarding *W. bancrofti* remains fairly stable over time.[46–49]

Hookworm infection in study participants was diagnosed by the examination of stool samples using the double-slide Kato–Katz method. In a manner similar to LF, we considered that individual HW infection status remains relatively stable over time in the absence of treatment. This assumption is based on the fact that 1) studies comparing egg densities before and after treatment in the same individuals suggest a predisposition to infection (and thus chronically exposed to a given level of exposure. Hence, the result of the earliest particularly in women)[50–54] and 2) intensity of exposure to HW is closely related to environmental factors,[51,55] and one can assume that, once married, females living in rural African areas remain more or less in the same perimeters of activities; stool examination performed during the course of the trial was used in the analyses presented here.

**Other independent variables.** Before inclusion in the albendazole community trial, participants were asked whether they had ever taken anthelminthic drugs before commencement of the trial in 2014. In the statistical analysis, we used the history of anthelmintic treatment (HAHT) before 2014 as a binary variable (yes versus no). Participants were grouped into four balanced age categories (< 31, 31–35, 36–44, and ≥ 45 years), with the number of deliveries analyzed using four categories for the bivariate analysis (< 4, 4–5, 6–7, and ≥ 8), and two categories for the logistic regression (< 5 versus ≥ 5) because of the small sample size.

**Statistical analysis.** Each independent quantitative variable was described by its arithmetic mean, SD, median, and interquartile range. Each independent qualitative variable was described using percentages (of total number of women). Bivariate analyses relating LF and HW infection status to demographic and reproductive variables were performed by $chi^2$ or Fisher's exact test if $chi^2$ conditions were not respected. For quantitative variables, nonparametric mean comparisons (Mann–Whitney test) were performed. Qualitative ordinal variables were analyzed using Cuzick's test for trend. In a second step, multivariable logistic regression was used to assess the association between dependent and independent variables while adjusting for potential confounders. Four dependent variables were analyzed using logistic regressions: postpartum hemorrhage, miscarriage, neonatal mortality, and preterm birth, expressed as binary variable (history of adverse outcome or no history of adverse outcome). For logistic regression, we included all variables, whether significant or not; that is, we used saturated models. Some of the questionnaires were not fully completed by the nurse in charge of its application, resulting in missing data. For all models, the significance of relevant interaction terms was assessed (age and infection status, age and HAHT, infection status, and HAHT). For variables with missing data included in the logistic regression, we created a "missing data" category. All statistical analyses were performed using STATA 15.1 (StatCorps, College Station, TX).

## RESULTS

**Study population.** A total of 215 women participated in the parasitological examinations conducted as part of the albendazole trial in 2018, and 113 (52.5%) were at least 15 years old. Of the 113 eligible women, 12 (10.6%) women refused to participate in the study. Thirteen were excluded because they were single or had lived as a couple for less than 2 years. Six others were excluded from the analysis because of ambiguity in the responses or because the questionnaires contained only sociodemographic information. A total of 82 women were thus included in the analysis. Only two of the participants have had no children. Seventy-two of them (87.8%) had participated in the first albendazole distribution in 2014 and were followed up until 2018. Ten others were included in the cohort during subsequent years (five in 2015, three in 2016, and two in 2018). Table 1 summarizes the information regarding the sociodemography and the variables related to reproductive health in the study population. The mean age of the participants was 37 years (range: 17–74). By comparison to the 82 women included in the analyses, those 13 who have been excluded were significantly younger (mean age: 25 years, $P < 0.001$) but did not differ in terms of HW or LF infection prevalence ($P = 0.135$ and 0.770, respectively).

TABLE 1
Description of main sociodemographic variables

| Variable | |
|---|---|
| Age (years) | |
| Mean (SD) | 37 (11.7) |
| Median (IQR) | 35 [30–44] |
| Already gave birth, *n* (%) | |
| Yes | 80 (97.6) |
| No | 2 (2.4) |
| Number of deliveries | |
| Mean (SD) | 5.2 (2.6) |
| Median (IQR) | 5 [3–7] |
| Currently pregnant, *n* (%) | |
| Yes | 10 (16.4) |
| No | 51 (83.6) |
| History of postpartum hemorrhage | |
| Yes | 21 (36.2) |
| No | 37 (63.8) |
| Number of postpartum hemorrhage | |
| Mean (SD) | 1.15 (2.23) |
| Median (IQR) | 2 [1–5] |
| History of miscarriage, *n* (%) | |
| Yes | 28 (43.7) |
| No | 36 (56.3) |
| Number of miscarriages | |
| Mean (SD) | 0,8 (1.2) |
| Median (IQR) | 1 [1–2] |
| History of neonatal mortality, *n* (%) | |
| Yes | 45 (56.2) |
| No | 35 (43.8) |
| History of preterm birth | |
| Yes | 12 (22.6) |
| No | 41 (77.4) |
| Number of preterm births | |
| Mean (SD) | 0.32 (0.75) |
| Median (IQR) | 1 [1–1] |
| History of anthelminthic treatment before the first albendazole treatment given as part of the trial, *n* (%) | |
| Yes | 51 (62.2) |
| No | 31 (37.8) |

IQR = interquartile range. For reproductive health variables, means were calculated on all the participants and medians only on those who experienced the event.

**Parasitological characteristics and bivariate analyses.** Thirty-four of the 82 women included in the analyses (44.5%) were positive by the FTS test before their first albendazole treatment. Only 60 (45 in 2014 and 15 in 2015) women provided a stool sample for examination; HW infection rate was 43.3% (26/60). Table 2 shows the main reproductive health variables considered here, stratified according to the participants' LF infection status. The presence of filarial antigenemia was not associated with the history of miscarriage ($P = 0.847$), history of preterm birth ($P = 0.730$), history of postpartum hemorrhage ($P = 0.587$), history of neonatal mortality ($P = 0.264$), or with the number of deliveries ($P = 0.772$). Table 3 shows the main reproductive health variables, stratified according to HW infection status. Hookworm infection was not associated with the history of miscarriage ($P = 1.000$), history of premature birth ($P = 0.670$), history of postpartum hemorrhage ($P = 0.294$), history of neonatal mortality ($P = 0.531$), or with the number of deliveries ($P = 0.396$). The number of deliveries (expressed as a continuous variable) was not associated with HW infection (5.54 deliveries in infected women versus 4.60 in noninfected, in mean; $P = 0.141$, Mann–Whitney test) nor LF infection (5.72 deliveries in infected women versus 4.97 in noninfected, in mean; $P = 0.158$, Mann–Whitney test).

**Multivariable logistic analyses among pregnancy adverse outcomes, LF and HW infections, and history of anthelminthic treatment.** The results of multivariable logistic regressions to assess the effect of LF and HW infections on the incidence of four pregnancy adverse outcomes are summarized in Table 4. History of neonatal mortality was more frequent in women with a positive FTS, but this was not significant ($P = 0.135$). Hookworm infection had no impact on any of the four main outcomes. Increase in age was strongly correlated with increase in miscarriage and neonatal mortality histories. Age was not included in the preterm birth model because of convergence issues. The number of deliveries was correlated with prematurity (adjusted odds ratio (aOR) = 11.6, $P = 0.008$ for women who had more than five deliveries with less than five deliveries as the reference category). Neonatal mortality was significantly less frequent in women with a HAHT (aOR = 0.2 with 95% CI = [0.04–0.82], $P = 0.024$).

## DISCUSSION

The aim of the present study was to document, for the first time, pregnancy outcomes in a rural population of DRC, and assess whether pregnancy outcomes were related to HW or LF parasitological status. No significant associations were found between infections with *W. bancrofti* or with HW, and the four pregnancy outcomes we focused on (miscarriage, preterm birth, neonatal mortality, and postpartum hemorrhage). The only significant result was that women with HAHT before the first albendazole treatment given as part of the trial had a significantly lower frequency of history of neonatal mortality.

The significant relationship observed here between HAHT and neonatal mortality is intriguing but will require further exploration to confirm and establish whether it is due to biological causes such as anemia (see in the following text) or is instead a product of other factors such as sociological determinants. Despite the potential benefits of anthelmintic treatment among pregnant women and an informal consultation made by the

TABLE 2
Distribution of sociodemographic and reproductive health variables according to the FTS result (presence or absence of *Wuchereria bancrofti*)

| Variable | Total | FTS- | FTS+ | *P*-value |
|---|---|---|---|---|
| Age-group (years), *n* (%) | | | | 0.111 |
| < 31 | 22 (26.8) | 11 (50.0) | 11 (50.0) | |
| 31–35 | 20 (24.4) | 16 (80.0) | 4 (20.0) | |
| 36–44 | 20 (24.4) | 12 (60.0) | 8 (40.0) | |
| ≥ 45 | 20 (24.4) | 9 (45.0) | 11 (55.0) | |
| Age (continuous) | | | | 0.394 |
| Mean (SD) | 35.30 (12.49) | 33.98 (11.02) | 37.12 (14.21) | |
| Current pregnancy, *n* (%) | | | | 0.524 |
| Yes | 10 (16.4) | 6 (60.0) | 4 (40.0) | |
| No | 51 (83.6) | 28 (54.9) | 23 (45.1) | |
| MD | 21 | 14 | 7 | |
| First trimester miscarriage, *n* (%) | | | | 0.574 |
| Yes | 14 (28.0) | 9 (64.3) | 5 (35.7) | |
| No | 36 (72.0) | 20 (55.6) | 16 (44.4) | |
| MD | 32 | 19 | 13 | |
| Miscarriage, *n* (%) | | | | 0.847 |
| Yes | 28 (43.7) | 17 (60.7) | 11 (39.3) | |
| No | 36 (56.3) | 21 (58.3) | 15 (41.7) | |
| MD | 18 | 10 | 8 | |
| Preterm birth, *n* (%) | | | | 0.730 |
| Yes | 12 (58.3) | 7 (58.3) | 5 (41.7) | |
| No | 41 (77.4) | 28 (68.3) | 13 (31.7) | |
| MD | 29 | 13 | 16 | |
| Postpartum hemorrhage, *n* (%) | | | | 0.587 |
| Yes | 21 (36.2) | 14 (66.7) | 7 (33.3) | |
| No | 37 (63.8) | 22 (59.5) | 15 (40.5) | |
| MD | 24 | 12 | 12 | |
| Neonatal mortality, *n* (%) | | | | 0.264 |
| Yes | 45 (56.2) | 24 (53.3) | 21 (46.7) | |
| No | 35 (43.8) | 23 (65.7) | 12 (34.3) | |
| MD | 2 | 1 | 1 | |
| Number of deliveries (categories), *n* (%) | | | | 0.772 |
| < 4 | 24 (30.0) | 15 (62.5) | 9 (37.5) | |
| 4–5 | 19 (23.7) | 12 (63.2) | 7 (36.8) | |
| 6–7 | 22 (27.5) | 13 (59.1) | 9 (40.9) | |
| ≥ 8 | 15 (18.8) | 7 (46.7) | 8 (53.3) | |
| MD | 2 | 1 | 1 | |
| Number of deliveries (continuous) | | | | |
| Mean (SD) | 5.29 (2.60) | 4.97 (2.61) | 5.72 (2.56) | 0.158 |

FTS = Filarial Test Strip; MD = missing data. For binary outcomes, the chi$^2$ test has been used (or Fisher's exact test if chi$^2$ conditions were not respected). For quantitative variables, the Mann–Whitney test has been performed.

WHO[56] which recommended treatment of all pregnant women with praziquantel and albendazole in areas endemic for STH or schistosomiasis, widespread adoption has not been observed. To date, only a minority of countries have included anthelmintic intake (albendazole or mebendazole) during routine pregnancy care, in the second and third trimesters of pregnancy: specifically, Madagascar, Nepal, and Sri Lanka.[57] Published studies suggest that it may be because of a fear of adverse birth outcomes due to a lack of safety data. More studies are needed to assess the benefits of implementing systematic deworming for pregnant women in resource-poor settings.

One of the main limitations of this study is the lack of power because of the small number of women recruited and the missing data in the questionnaires which were frequently incorrectly completed. An additional limitation is that pregnancy outcomes were analyzed using infection as a binary indicator (presence/absence) rather than a continuous one (based on the intensity of infection measured by, e.g., the FTS score for LF or egg counts per gram of feces for HW). This stratification was undertaken because of limitations in the sample size.

Another important limitation of our study is that we did not collect hemoglobin levels from the participating women. Indeed, it is well established that HW infection can cause anemia.[58] As well, pregnant women are more prone to anemia because of the physiological changes that typically accompany pregnancy.[59] It is likely then that pregnant women infected with HW are a population at high risk for anemia. Anemia has been implicated in the occurrence of adverse pregnancy events such as perinatal and neonatal mortality,[60] prematurity, and low birth weight.[61] Indeed, work from Mpairewe et al.[39] has shown that infection with helminths such as HW are associated with anemia in pregnant women, and that the risk of anemia was highly correlated with the infection intensity. A final limitation is that we collected retrospective data, and therefore, these data are subject to recall bias.

Our study was a retrospective observational study, but to assess more specifically the relationships between parasitic infections and pregnancy outcomes, prospective cohort studies are required. Examples of such studies are present in the literature: Christian et al.[62] conducted a clinical trial of albendazole (zero, one, or two doses) among 4,998 pregnant women in Nepal coinfected by the three geohelminths (prevalence of HW: 47.5%). They assessed the proportion of severe anemia in the treated (one or two doses) and untreated groups and found that 20% of the women belonging to the latter

TABLE 3
Distribution of sociodemographic and reproductive health variables according to the presence or absence of HW at the Kato–Katz stool examination

| Variable | Total | HW- | HW+ | *P*-value |
|---|---|---|---|---|
| Age-group (years), *n* (%) | | | | 0.098 |
| < 31 | 19 (31.7) | 11 (57.9) | 8 (42.1) | |
| 31–35 | 13 (21.7) | 9 (69.2) | 4 (30.8) | |
| 36–44 | 14 (23.3) | 10 (71.4) | 4 (28.6) | |
| ≥ 45 | 14 (23.3) | 4 (28.6) | 10 (71.4) | |
| Age (continuous) | | | | |
| Mean (SD) | 34.91 (13.11) | 33.14 (10.57) | 36.91 (15.42) | 0.461 |
| Current pregnancy, *n* (%) | | | | 1 |
| Yes | 8 (18.6) | 4 (50.0) | 4 (50.0) | |
| No | 35 (81.4) | 20 (57.1) | 15 (42.9) | |
| MD | 17 | 10 | 7 | |
| First trimester miscarriage, *n* (%) | | | | 1 |
| Yes | 7 (21.2) | 5 (71.4) | 2 (28.6) | |
| No | 26 (78.2) | 16 (61.5) | 10 (38.5) | |
| MD | 27 | 13 | 14 | |
| Miscarriage, *n* (%) | | | | 1 |
| Yes | 13 (30.9) | 8 (61.5) | 5 (38.5) | |
| No | 29 (69.1) | 19 (65.5) | 10 (34.5) | |
| MD | 18 | 7 | 11 | |
| Preterm birth, *n* (%) | | | | 0.670 |
| Yes | 6 (16.2) | 3 (50.0) | 3 (50.0) | |
| No | 31 (83.8) | 19 (61.3) | 12 (38.7) | |
| MD | 23 | 12 | 11 | |
| Postpartum hemorrhage, *n* (%) | | | | 0.294 |
| Yes | 12 (31.6) | 9 (75.0) | 3 (25.0) | |
| No | 26 (68.4) | 14 (53.8) | 12 (46.2) | |
| MD | 22 | 11 | 11 | |
| Neonatal mortality, *n* (%) | | | | 0.531 |
| Yes | 31 (53.5) | 17 (54.8) | 14 (45.2) | |
| No | 27 (46.5) | 17 (63.0) | 10 (37.0) | |
| MD | 2 | 0 | 2 | |
| Number of deliveries (categories), *n* (%) | | | | 0.396 |
| < 4 | 18 (31.0) | 13 (72.2) | 5 (27.8) | |
| 4–5 | 16 (27.6) | 9 (56.3) | 7 (43.7) | |
| 6–7 | 16 (27.6) | 8 (50.0) | 8 (50.0) | |
| ≥ 8 | 8 (13.8) | 3 (37.5) | 5 (62.5) | |
| MD | 2 | 1 | 1 | |
| Number of deliveries (continuous), *n* (%) | | | | |
| Mean (SD) | 5.00 (2.44) | 4.60 (2.45) | 5.54 (2.38) | 0. 141 |

MD = missing data; HW = hookworm.
For binary outcomes, the $chi^2$ test has been used (or Fisher's exact test if $chi^2$ conditions were not respected). For quantitative variables, the Mann–Whitney test has been performed.

suffered of severe anemia (hemoglobin < 70 g/L) versus 5% in the treated group. There was also evidence of an impact on infant mortality (i.e., mortality in the 6 months after birth), which was 14% lower in the one-dose group and 41% lower in the two-dose group than those in the untreated group. In a similar clinical trial conducted in 2006 in Peru among 1,042 women in the second trimester of pregnancy and coinfected by the three geohelminths, Larocque et al.[63] compared the proportion of anemia in a group treated with placebo and iron supplementation and a group treated with mebendazole (500 mg) and iron supplementation. These authors did not observe a significant difference in anemia proportion at the third trimester, but this could be due to the iron supplementation provided to both groups. However, the study did show that the proportion of very low weight birth infants was higher in the placebo group than that in the mebendazole group.[64] Although the results appear to be suggestive of impacts of geohelminths on adverse pregnancy outcomes, a limitation of both the studies described earlier is that the effect of STH infection on pregnancy outcome was assessed indirectly, through administration of anthelminthic treatment, rather than directly. A number of other parasites sensitive to the same treatments (*Taenia* spp., *E. vermicularis*, *Strongyloides stercoralis* . . .) and which frequently co-occur with STHs could therefore be implicated. To our knowledge, the only prospective cohort study exploring the direct implication of HW on the pregnancy outcomes is a Bolivian longitudinal 9-year study conducted in an area with high prevalence of HW. It showed that HW infection was associated with lower body mass index of the women, lower number of deliveries, and an older age at first pregnancy.[22] Some cross-sectional studies have examined the occurrence of adverse pregnancy events according to the HW infection status. Two of the four published studies on the relationship between HW and infant mortality found an association,[65,66] whereas the other two found no association.[67,68] Results in the literature are similarly mixed for the relationship between HW infection and premature birth; Wanyonyi et al.[66] and Asundep et al.[65] found an association, whereas Mahande and Mahande[68] did not.

For LF infection, despite the fact that we did not find any significant association with pregnancy outcomes, our results suggest that LF may have an implication on the neonatal mortality. Because of the small number of women recruited, this possible association has to be reevaluated within a larger study, and using a more suitable design such as a prospective cohort study with recurrent assessments of infection levels, hemoglobin levels, and adverse pregnancy events. A case report

TABLE 4
Logistic regression analysis on the four dependent variables: miscarriage, preterm birth, neonatal mortality, and postpartum hemorrhage

| | (1) | | (2) | | (3) | | (4) | |
|---|---|---|---|---|---|---|---|---|
| | Miscarriage | | Preterm birth | | Neonatal mortality | | Postpartum hemorrhage | |
| | OR (95% CI) | *P*-value | OR (95% CI) | *P*-value | OR (95% CI) | *P*-value | OR (95% CI) | *P*-value |
| *Wuchereria bancrofti* | | | | | | | | |
| Absence | Ref. | | Ref. | | Ref. | | Ref. | |
| Presence | 0.7 (0.2–2.7) | 0.562 | 1.7 (0.3–8.9) | 0.544 | 2.8 (0.7–10.7) | 0.135 | 0.4 (0.1–1.6) | 0.187 |
| Hookworm | | | | | | | | |
| Absence | Ref. | | Ref. | | Ref. | | Ref. | |
| Presence | 0.7 (0.1–3.4) | 0.612 | 1.4 (0.2–11.0) | 0.739 | 1.3 (0.3–5.4) | 0.698 | 0.3 (0.1–1.8) | 0.199 |
| Missing data* | 4.2 (0.9–19.1) | 0.061 | 4.9 (0.6–38.9) | 0.134 | 1.5 (0.3–6.8) | 0.606 | 1.6 (0.4–6.9) | 0.494 |
| Age-group (years) | | | Not included† | | | | | |
| < 30 | Ref. | | | | Ref. | | Ref. | |
| 30–35 | 1.6 (0.2–11.0) | 0.622 | | | 16.2 (2.4–108.3) | 0.004 | 0.3 (0.0–1.7) | 0.165 |
| 36–44 | 3.9 (0.5–28.9) | 0.185 | | | 29.9 (3.2–281.7) | 0.003 | 0.4 (0.0–3.0) | 0.350 |
| ≥ 45 | 14.9 (1.5–150.8) | 0.022 | | | 24.2 (2.5–233.4) | 0.006 | 0.5 (0.1–5.0) | 0.591 |
| Number of deliveries | | | | | | | | |
| < 5 | Ref. | | Ref. | | Ref. | | Ref. | |
| ≥ 5 | 1.0 (0.2–4.2) | 0.976 | 11.6 (1.9–70.5) | 0.008 | 1.8 (0.4–7.8) | 0.437 | 2.6 (0.5–13.5) | 0.250 |
| History of anthelmintic treatment | | | | | | | | |
| No | Ref. | | Ref. | | Ref. | | Ref. | |
| Yes | 0.4 (0.1–2.0) | 0.285 | 0.1 (0.01–1.5) | 0.106 | 0.2 (0.0–0.8) | 0.024 | 0.9 (0.2–3.7) | 0.894 |
| Missing data* | 0.9 (0.1–5.5) | 0.883 | 0.5 (0.1–4.7) | 0.555 | 0.3 (0.1–2.1) | 0.253 | 0.3 (0.1–2.0) | 0.226 |
| Number of observations‡ | 64 | | 53 | | 79 | | 58 | |

OR = odds ratio; Ref. = reference category.
* Missing data were included in the regression model as a categorical variable to improve convergence and number of participants.
† Age could not be included in prematurity logistic regression because of convergence issue due to the relatively small number of participants.
‡ Number of observations are smaller than 82 because of missing information in the questionnaires administrated to women or missing data in parasitological results.

published in 2006 showed that an LF infection in a 36-year-old woman may have led to implantation failure in in vitro fertilization cycles.[69] If LF infection can cause implantation issue during in vitro fertilization cycles, one cannot exclude that the parasite may cause pregnancy outcome issues and fertility issues.

This study describes the frequency of adverse pregnancy events and LF and HW infections in a rural population of the DRC. Although no significant associations were found between infections with *W. bancrofti* or with HW, and miscarriage, preterm birth, neonatal mortality and postpartum hemorrhage, women with HAHT had significantly less frequent history of neonatal mortality. This topic of research is under-explored, and more studies are needed to understand whether HW and LF are involved in the occurrence of adverse pregnancy events.

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## Abstract

Onchocerciasis and lymphatic filariasis are both parasitic diseases that are targeted for elimination by control programs in Central Africa. These programs are based on massive drug administration to the entire population living in endemic areas. The choice of drugs used is based on the endemicity of onchocerciasis and lymphatic filariasis. Thus, ivermectin is used in areas where only onchocerciasis is endemic, while the combination of ivermectin and albendazole is used in areas where lymphatic filariasis and onchocerciasis are coendemic.

These treatments must be repeated because they kill the larval stage of the parasite, the microfilariae, but not the adult worms. These control programs are undoubtedly successful, but the fight continues. A current issue is the use of ivermectin in areas where another filariasis, loaisis, is also endemic. Indeed, subjects with a high microfilarial density of L*oa loa* in the blood are at risk of serious post-ivermectin adverse effects. Although the benefit/risk ratio of mass ivermectin treatment remains acceptable in these areas when onchocerciasis or lymphatic filariasis are meso-hyperendemic, it is not acceptable when they are hypoendemic.

In order to interrupt the transmission of lymphatic filariasis and onchocerciasis, it is essential to develop effective and safe alternative strategies to control these filariasis in areas where they are hypoendemic and coendemic for loaisis, and to accelerate the control of these filariasis in areas where they are meso- or hyperendemic and coendemic for loaisis. Finally, in order to treat these areas, it seems important to better understand loaisis, which has been ignored by public health programs.

This work provides new evidence for the evaluation of an existing alternative strategy: the semi-annual administration of albendazole against lymphatic filariasis in areas where loaisis is endemic. The added value of maintaining good compliance with mass albendazole administration programs has been evaluated.

This work also reports the results of a clinical trial investigating the use of levamisole to decrease *Loa loa* microfilaremia below the threshold for the occurrence of severe post-ivermectin adverse effects, and thus would allow the safe administration of ivermectin to the general population in control programs.

New approaches have also been investigated: the prophylactic effect of ivermectin which could be of interest in accelerating control through more frequent mass treatments and as chemoprophylaxis for travelers and expatriates.

The use of levamisole in the management of loaisis or of ivermectin as chemoprophylaxis for onchocerciasis requires a precise evaluation of the safety of these molecules. We have thus conducted two pharmacovigilance studies using the WHO global pharmacovigilance database.

Finally, concerning loaisis, we have studied and modeled the daily periodicity, the short- and long-term variability as well as the diagnostic variability of blood microfilaremia allowing to improve our understanding of the parasite and thus to bring new elements that will be useful in interventions seeking to evaluate new alternative strategies to manage endemic areas for loaisis

## Résumé

L'onchocercose et la filariose lymphatique sont toutes les deux des parasitoses faisant l'objet de programmes de lutte visant leurs éliminations en Afrique centrale. Ces programmes reposent sur l'administration massive de médicaments à toute la population vivant en zone d'endémie. Le choix des médicaments utilisés repose sur l'endémicité de l'onchocercose et de la filariose lymphatique. Ainsi, l'ivermectine est utilisée dans les zones où seule l'onchocercose est endémique tandis que l'association ivermectine et albendazole est utilisée dans les zones où la filariose lymphatique et l'onchocercose sont coendémiques.

Ces traitements doivent être répétés car ils tuent le stade larvaire du parasite, les microfilaires, mais pas les vers adultes. Ces programmes de lutte sont, sans aucun doute, une réussite mais la lutte continue. Une problématique qui se pose actuellement est l'utilisation d'ivermectine dans les zones où une autre filariose, la loase, est également endémique. En effet, les sujets ayant une forte densité microfilarienne à *Loa loa* dans le sang sont à risque d'effet secondaire grave post-ivermectine. Bien que le rapport bénéfice/risque des traitements de masse par ivermectine reste acceptable dans ces zones lorsque l'onchocercose ou la filariose lymphatique sont méso-hyperendémiques, il ne l'est pas en lorsqu'elles sont hypoendémiques.

Afin d'interrompre la transmission de la filariose lymphatique et de l'onchocercose, il est primordial de mettre en place des stratégies alternatives efficaces et sûres permettant de lutter contre ces filarioses dans les régions où elles sont hypoendémiques et coendémiques pour la loase et d'accélérer la lutte contre ces filarioses dans les régions où elles sont méso- ou hyperendémiques et coendémiques pour la loase. Enfin, pour parvenir à traiter ces zones, il semble important de mieux comprendre la loase, jusque-là ignoré par les programmes de santé publique.

Ces travaux apportent de nouveaux éléments concernant l'évaluation d'une stratégie alternative déjà existante : l'administration semi-annuel d'albendazole contre la filariose lymphatique dans les zones où la loase est endémique. La valeur ajoutée du maintien d'une bonne observance thérapeutique aux programmes d'administration de masse d'albendazole a été évaluée.

Ces travaux rapportent également les résultats d'un essai clinique portant sur l'utilisation du lévamisole pour diminuer la microfilarémie à *Loa loa* en dessous du seuil d'apparition des effets secondaires graves post-ivermectine, et ainsi permettrait l'administration sûre d'ivermectine à l'ensemble de la population dans le cadre des programmes de lutte.

De nouvelles pistes de recherche ont également été étudiées : l'effet prophylactique de l'ivermectine qui pourrait avoir un intérêt dans le cadre de l'accélération de la lutte par mise en place de traitements de masse plus fréquents et en tant que chimioprophylaxie préexposition à destination des voyageurs et des expatriés.

L'utilisation de lévamisole dans la prise en charge de la loase ou de l'ivermectine en chimioprophylaxie pour l'onchocercose nécessite d'évaluer précisément la sécurité d'emploi de ces molécules. Nous avons ainsi conduit deux études de pharmacovigilance à partir de la base mondiale de pharmacovigilance de l'OMS.

Enfin, concernant la loase, nous avons étudié et modélisé la périodicité journalière, la variabilité à court terme et à long terme ainsi que la variabilité diagnostique de la microfilarémie sanguine permettant de mieux comprendre le parasite et donc d'apporter des éléments qui seront utiles dans les interventions cherchant à évaluer de nouvelles stratégies alternatives pour prendre en charge les zones d'endémie pour la loase.