Trends in Parasitology

CellPress
REVIEWS

Review

The Population Biology and Transmission Dynamics of *Loa loa*

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Endemic to Central Africa, loiasis – or African eye worm (caused by the filarial nematode *Loa loa*) – affects more than 10 million people. Despite causing ocular and systemic symptoms, it has typically been considered a benign condition, only of public health relevance because it impedes mass drug administration-based interventions against onchocerciasis and lymphatic filariasis in co-endemic areas. Recent research has challenged this conception, demonstrating excess mortality associated with high levels of infection, implying that loiasis warrants attention as an intrinsic public health problem. This review summarises available information on the key parasitological, entomological, and epidemiological characteristics of the infection and argues for the mobilisation of resources to control the disease, and the development of a mathematical transmission model to guide deployment of interventions.

Highlights

Recently, the notion that loiasis is mostly a benign disease has been challenged. A cohort study in Cameroon showed a significant association between heavy *Loa loa* microfilaraemia and increased human mortality risk. Across Central Africa >10 million people are estimated to have loiasis.

Mathematical models of neglected tropical diseases (NTDs) can inform the implementation and evaluation of interventions aimed at controlling and eliminating these diseases. *Loa loa* is not among the 20 NTDs on the World Health Organization's list, and mathematical models for its transmission and control have never been formulated.

Little attention has been paid to assessing fundamental parasitological, entomological, and epidemiological processes that must be adequately quantified in mathematical models if these are to be developed as useful tools for optimal and cost-effective intervention design.

Loa loa: More Than Meets the Eye

The filarial nematode *Loa loa* (Cobbold, 1864), which is transmitted from human to human by tabanid flies (Diptera: Tabanidae) of the genus *Chrysops*, causes loiasis, a pathological condition often labelled as 'African eye worm'. Loiasis is endemic in heavily forested areas of Central Africa, including Cameroon, Democratic Republic of the Congo (DRC), Gabon, and Nigeria, where a combination of long adult worm lifespans (>20 years in some instances [1]) and continuous exposure to infective bites mean that individuals can harbour the worm for nearly their entire life. *L. loa* can cause both ocular and systemic symptoms; these include eye worm, caused by the migration of the worm under the **bulbar conjunctiva** (see Glossary), as well as subcutaneous oedemas known as Calabar swellings, which are thought to be caused by immunogenic reactions to the release of either **microfilariae** or antigenic material by adult worms [2]. Both manifestations are considered mild and without significant morbidity or impact on quality of life. More rarely, disease sequelae can prove more severe, including a variety of cardiac, renal, and neurological complications [3].

Despite this range of clinical manifestations, and that over 14 million people currently reside in high-risk areas [4], loiasis has typically been considered a benign condition and, in contrast to other filarial infections such as onchocerciasis and **lymphatic filariasis (LF)**, has not been included in the World Health Organization's list of **neglected tropical diseases (NTDs)** Appendix A. Recent research on the epidemiology of *L. loa* has been primarily concerned with the impediment it poses to the treatment of onchocerciasis and LF in individuals with high levels of *L. loa* microfilariae circulating in the bloodstream. These individuals have a greatly increased risk of developing **severe adverse events (SAEs)** if treated with ivermectin. Hence, in areas where loiasis is co-endemic with onchocerciasis and/or LF, the ability to safely carry out **mass drug administration (MDA)** with ivermectin (the cornerstone of onchocerciasis and LF control efforts in Africa) is impeded [5,6].

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Whilst the parasite's capacity to cause clinical disease has long been recognised, it is only recently that epidemiological studies have started to uncover the impact of loiasis on human morbidity and mortality. Most notably, a cohort study in an area of Cameroon (that has never been included in ivermectin MDA programmes) revealed a significant association between infection profiles characterized by high levels of *L. loa* microfilaraemia (microfilariae in the blood) and an increased relative risk of mortality [7]. Indeed, the population attributable fraction of mortality associated with loiasis was 14.5%, greater than the estimated 5% (across West Africa) caused by onchocerciasis [8]. This indicates that long-held conceptions of loiasis as a benign disease are misplaced, and that loiasis warrants attention as a public health issue in its own right, and not merely as a barrier to the treatment of other filarial diseases.

Mathematical transmission models of NTDs are increasingly being used to guide and quantify the impact of interventions aimed at controlling and eliminating NTDs and improving the health of many of the world's most impoverished populations. Yet for *L. loa*, no such models exist, and little attention has been paid to the fundamental population biological processes that underpin model development. Previous reviews have focused on entomological, clinical, and immunological aspects of loiasis [9,10], but a review summarising the pertinent epidemiological, population biology, and transmission dynamics features of the parasite remains outstanding. In the context of loiasis' newly uncovered public health importance [7], this review addresses this absence, making the case for increased interest and effort in researching the disease, and advocating for mobilisation of resources for its control using strategies guided by mathematical modelling.

Parasite Biology and Life Cycle

Adult *L. loa* reside in the layers of loose connective tissue beneath the skin, as well as between the fascial layers on top of somatic muscles [11], where they produce large numbers of embryonic progeny called microfilariae. Unlike other filariae, *L. loa* lacks *Wolbachia* endosymbionts [12,13], although it does not appear to have developed any novel specialised metabolic capabilities in compensation [14].

Microfilariae circulate in the peripheral blood during the daytime and, following ingestion during a bloodmeal, develop within the vector to infective (L3) stages during a process taking between 10 and 12 days (Figure 1). This duration of the **extrinsic incubation period (EIP)** is consistently reported in numerous studies conducted across Central Africa and across the two main species of *Chrysops* (*Chrysops silacea* and *Chrysops dimidiata*) responsible for human transmission [15,16], although the EIP is likely to vary with ambient temperature [17]. Indeed, there are reports of developmental times taking as long as 3–4 weeks in the cooler mountain valley regions of Cameroon (situated 4000 feet, i.e., ~1200 m above sea level) [18]. Whilst most research suggests that nearly all microfilariae ingested successfully develop into L3 larvae, and that there is little parasite mortality within the vector [3,9,19], only one experiment has actually been carried out to assess this, which concluded that 'the number of infective forms found in flies . . . is similar to the number of the microfilariae taken in' [20]. Our reassessment of these results, however, suggests that the actual proportion of ingested microfilariae progressing to L3 is lower, in the region of 40–50% (C. Whittaker, MSc thesis, Imperial College London, 2017). It is not known whether this proportion varies in a density-dependent manner with microfilarial intake, as has been observed for other filarial parasites such as *Onchocerca volvulus* [21] and *Wuchereria bancrofti* [22].

Upon inoculation into humans, L3 larvae undergo a third moult to become fourth-stage larvae (L4), and then a fourth and final moult to become young adult worms. After sexual maturation

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and development into fully reproductive adult worms, females produce microfilariae that periodically accumulate in the peripheral blood and are ingested by vectors during a bloodmeal. Estimates of the timings of these developmental processes are varied, with observed differences likely due to experiments having been conducted in different model organisms. In rodent animal models, the L3 to L4 transition occurs approximately 9 days after inoculation, with the transition from L4 to young adult taking place around day 20 [23]. In monkeys, by contrast, these transitions happen 16–20 and about 30 days after inoculation, respectively [24]. Despite this, microfilariae are not detected in the peripheral blood until 5 to 6 months later [11,25], implying some further delay in either their production (possibly caused by the time taken for adult sexual maturation and mating) or their release into the peripheral bloodstream from the lungs.

Fecundity and Microfilarial Production

Upon fertilisation, female worms produce microfilariae, each surrounded by a sheath formed from part of the initial egg shell. Studies of microfilarial production have been limited, but results of single-pair infections indicate that each female adult worm can produce between 12 000 and 39 000 microfilariae per day in the absence of reproductive constraints [26]. These estimates agree with our reanalysis (Figure 2) of similar data involving experimentally infected primates [25], in which we have estimated fecundity, microfilarial lifespan, and rate of progression from inoculated L3 larvae into reproductively active female worms. Differences in these estimates were observed across data from different animal models, including patas monkeys (*Erythrocebus patas*, not a natural host; Figure 2A) and baboons (*Papio anubis*, a natural host; Figure 2B). Estimates of adult worm lifespan are rare, but the average (life expectancy) is thought to be at least 9 years [25], with reports of some worms surviving for as long as 15–21 years [1,27,28].

A non-linear relationship appears to exist between the number of adult worms an individual harbours and the observed intensity of infection with microfilariae, with single-pair infections capable of producing concentrations of microfilariae in the blood similar to those of infections composed of several pairs of worms [25]. Such variability in infection intensities has also been observed in more recent work, where a number of baboons, all infected with 600 L3 larvae, displayed as much as a 50-fold difference in their resulting microfilarial loads [29] (such a phenomenon is similar to that observed for *Ascaris suum* in experimentally and naturally infected pigs [30]). There is, however, some positive correlation between the number of worms harboured and the resulting concentration of microfilariae in the blood, with other research also involving experimental infection of primates demonstrating that, whilst monkeys inoculated with 200 L3 larvae had, on average, higher concentrations of microfilariae than those inoculated with 75 L3 larvae, there was significant overlap between infection intensities observed across the two groups [25]. The results are, therefore, suggestive of some non-linear (and possibly density-dependent) relationship between worm burden and microfilaraemia, a phenomenon reminiscent of that directly observed for *Ascaris lumbricoides* [31] and suggested for *O. volvulus* [32] (although, in the latter case, other studies have failed to substantiate these findings [33]). Although it is apparent that worm burden and microfilarial intensity are not linearly related, it remains to be established whether this is a product of density-dependent processes acting on female worm fecundity, or on other aspects of parasite or host biology.

Of the microfilariae produced by adult worms, only a small proportion are present at any given time in the peripheral blood and become available for ingestion during a bloodmeal by *Chrysops* species vectors. Some estimates have used a value of 10%, based on studies conducted using the dog heartworm *Dirofilaria immitis* [26,34], whereas others have estimated it directly as

Glossary

Amicrofilaraemic: a term used to denote individuals with no detectable levels of microfilariae in their blood.
Bulbar conjunctiva: the portion of the conjunctiva (a clear thin membrane that covers the front surface of the eye) covering the 'whites' of the eye.
Diethylcarbamazine (N,N-diethyl-4-methyl-1-piperazine carboxamide, DEC): a medication used to treat lymphatic filariasis in endemic areas other than those in Africa as it is contraindicated in onchocerciasis.
Extrinsic incubation period (EIP): in vector-borne diseases, the interval between the acquisition of the stages infectious to the vector and the transmission of the stages infective to other susceptible vertebrate hosts. As arthropod vectors are poikilotherms, the EIP depends on temperature.
Lymphatic filariasis (LF): commonly known as elephantiasis, LF is a neglected tropical disease caused by infection with lymphatic filariae (*Wuchereria bancrofti*, *Brugia malayi*, *Brugia timori*) transmitted to humans through the bites of mosquitoes.
Mass drug administration (MDA): the treatment, with a chemotherapeutic agent, of a population or population group considered to be at risk of infection in a geographical area without first diagnosing the infection, and regardless of the presence of symptoms.
Maximum likelihood: a mathematical method for estimating the parameters of a statistical model when such a model is fitted to data. The likelihood is the probability of observing a particular set of sample data given a particular statistical model and parameters. Maximum likelihood estimation involves finding the values of the parameter(s) that 'maximise' the likelihood of observing the data.
Microfilariae: an early stage in the life cycle of *Loa loa* and other filarial nematodes. Produced by reproductively active adult females, this embryonic stage of the parasite circulates in the peripheral blood of humans and is taken up during blood feeding by the *Chrysops*

between 5% and 20% based on experimental infections in primate species and comparison of microfilarial concentrations in pulmonary and peripheral blood [11].

Whilst previous historical estimates of microfilarial lifespan placed it in the region of 6–12 months [11], this was primarily based on empirical observations of the time taken for microfilarial counts to stabilise and plateau in infected primates. Our analysis of more recently published data [24] suggests that the average lifespan is shorter, between 3 and 5 months (Figure 2C).

Vector Biology and Life Cycle

Chrysops Species Vectors of *L. loa*

In humans, *L. loa* is transmitted primarily by two species of the tabanid family, *C. silacea* and *C. dimidiata*. They are found across the tropical rainforests of Central and Western Africa, alongside other *Chrysops* species, including *Chyrsops langi, Chrysops centurionis*, *Chrysops zahrai* and *Chrysops longicornis*. These species are not considered to be primary vectors of the human strain of *L. loa*, and instead are thought to contribute to the maintenance of the simian form of the infection (discussed below) through their crepuscular/nocturnal biting habits (simian *L. loa* exhibits nocturnal periodicity of microfilarial circulation in the peripheral blood), and preference for primate bloodmeals [10]. Whilst *C. silacea* has been more abundant than *C. dimidiata* at many of the sites studied [35], others have observed *C. dimidiata* to be more abundant [36], highlighting the importance of local ecological factors in underpinning species' abundance. Both species exhibit a strong annual variation in abundance, peaking during the rainy season for *C. silacea*, and somewhat earlier for *C. dimidiata* [37].

Biting Patterns and Transmission

C. silacea and *C. dimidiata* display similar feeding patterns and host preferences. Both species show strong diurnal biting preferences, with biting activity being greatest during the morning (approximately 9–11 a.m.) and late afternoon (approximately 2–4 p.m.), coinciding with the appearance of *L. loa* microfilariae in the blood of infected humans, with microfilaraemia in infected individuals typically peaking somewhere between 10 a.m. and 4 p.m. [38,39]. It is estimated that both species bite once every 5 days [40], coherent with the estimates of 5–6 days for the duration of the gonotrophic cycle [41,42]. Biting frequency appears somewhat variable, however, and has been shown to increase with temperature but decline with relative humidity [38]. Unlike other flies, both species are heavily attracted by the smoke of wood fires, which significantly increases biting densities [43], although the species appear differentially sensitive to it as an attractant (with *C. silacea* biting densities increasing 11-fold and *Chrysops dimidiata* 4.5-fold [44]). Humans constitute the vector's main blood source; approximately 90% of bloodmeals are taken on them, with a mixture of wild animals (not including primates, limiting the capacity for cross-transmission) making up the remainder [45]. Whilst historically it has been suggested that the biting habits of parous and nulliparous flies differ, with parity determining whether flies bite in the morning or late afternoon [46], more recent work has not confirmed such a difference, and it appears that the biting habits of nulliparous and parous flies are broadly similar [39].

Estimates of average bloodmeal size (a key determinant along with host microfilarial levels of the number of microfilariae ingested) vary across the literature, depending on a number of factors including whether flies were allowed to feed to repletion and whether the study was conducted in a laboratory or in a natural setting. The typical bloodmeal size in *C. silacea*, when allowed to feed to repletion under laboratory conditions, is about 40 mg [47,48], varying between 25 and 55 mg. Under natural conditions, bloodmeal size in both *C. silacea* and *C. dimidiata* is smaller,

horsefly species that act as vectors of the parasite.

Neglected tropical diseases (NTDs): an umbrella term encompassing a diverse group of (mostly) communicable diseases of poverty (excluding malaria, HIV, and TB), which was coined against the backdrop of these 'big three' to raise the profile of, and unite efforts against tropical/sub-tropical infections/conditions. NTDs are particularly prevalent in low- and middle-income populations in developing regions of Africa, Asia, and the Americas. They are caused by a variety of pathogens, including viruses, bacteria, protozoa and helminths.

Overdispersion: in statistics and parasitology, the presence of greater variability (statistical dispersion around the mean) than would be expected based on a given (generally Poisson) distribution. As in the Poisson (random) distribution, the variance is equal to the mean (the variance to mean ratio, VMR, is equal to 1), an overdispersed distribution has a VMR >>1. The number of parasites per host is more often than not overdispersed.

Severe adverse event (SAE): any untoward medical occurrence during a drug clinical trial or following an approved treatment that: results in death; is life-threatening; requires inpatient hospitalization or causes prolongation of existing hospitalization; results in persistent or significant disability/incapacity; leads to a congenital anomaly/birth defect, or requires intervention to prevent permanent impairment or damage.

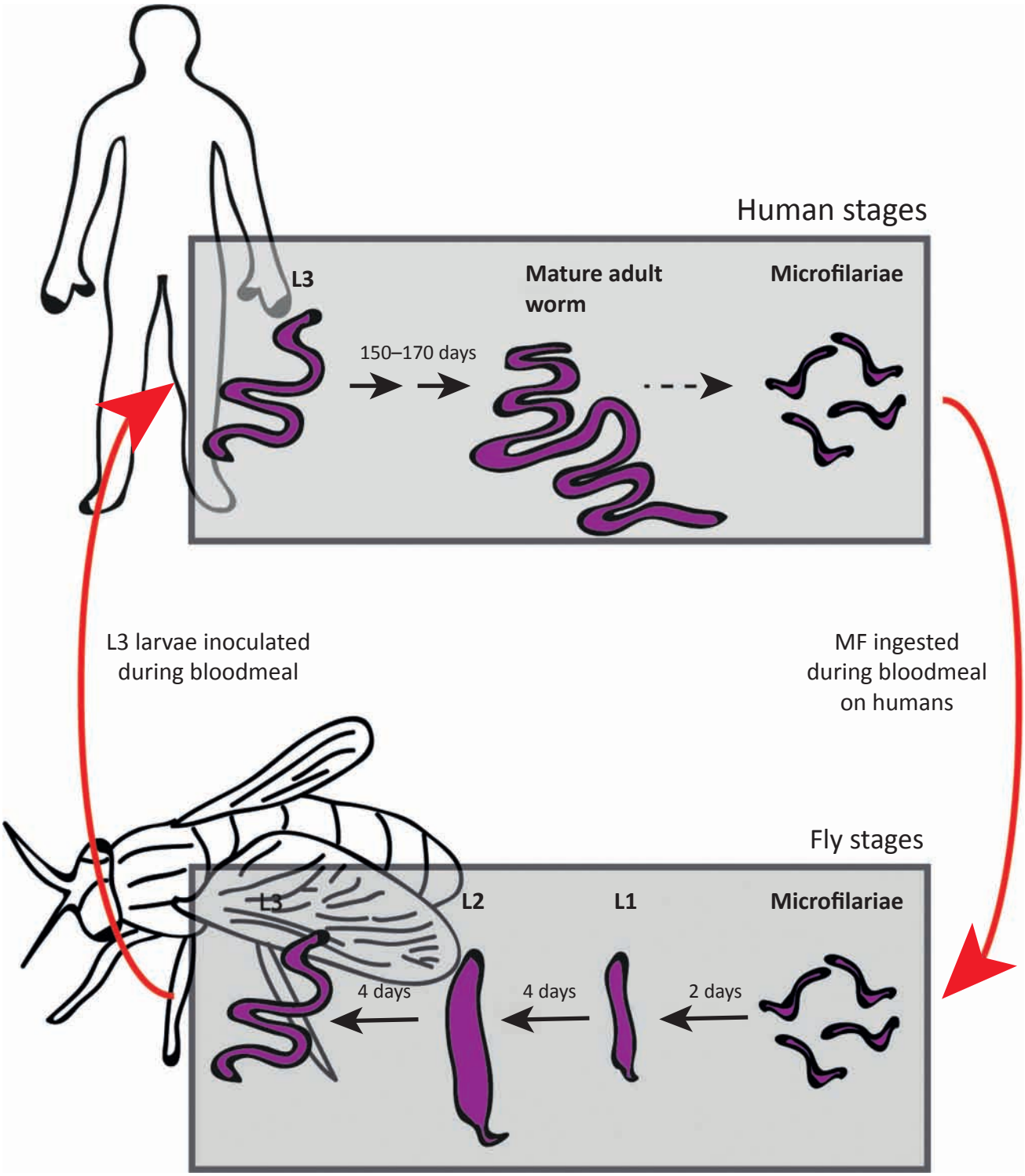

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Figure 1. The Life Cycle and Development of *Loa loa*. Adult *L. loa* reside in the subcutaneous tissue of the human host, where males and females mate and produce microfilariae. Microfilariae (MF) accumulate in the pulmonary blood and periodically in the peripheral blood, where they can be transmitted to tabanid vectors

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averaging 25 mg and 20 mg, respectively [19]. Estimates of the frequency with which *Chrysops* species feed to repletion in the wild are lacking, although feeding to repletion is not necessary for ovary development, with bloodmeals above 14 mg shown to be sufficient for normal development of both the ovaries and any ingested *L. loa* microfilariae [42]. The actual bite itself is not painful, with the often reported pain of the bite actually associated with the withdrawal of biting parts upon completion of the bloodmeal [16]. Thus, many hosts usually notice the bite only after the fly has finished feeding. It's more common, perhaps, for individuals to notice the landing of the fly and brush it away before feeding commences, a possible consequence of their large size and striking patterned colour [49]. One report from Gabon, for example, suggests that the proportion of landings that successfully translates into bloodmeals is 14% [50].

It has been noted that the intake of microfilariae in flies fed on infected individuals is lower than would be expected based on the size of the bloodmeal and the concentration of microfilariae in the host's blood [48]. This might be a product of the mechanism by which *Chrysops* feeds, which involves feeding from a pool of blood produced by laceration [51], rather than from a capillary, as is common for other species of blood-feeding insects (particularly mosquitoes). It has been postulated that there might be a delay in the accumulation of microfilariae in this pool, such that the fly ingests a smaller amount than would be expected.

Vector Mortality, Competence, and Carriage of L3 Larvae

Microfilariae are thought to develop into L3 larvae in the fat body of the fly [52] before dispersing widely throughout the body of *Chrysops*, although strictly emerging from the fly's head when it takes a bloodmeal. It is suggested that these larvae are able to move freely between the body and the head, such that L3 larvae from other parts of the body can be recruited to the head during times of feeding [51].

The emergence of L3 larvae from the vector's head is thought to incur a significant amount of excess mortality, something perhaps not surprising given the large number of larvae that flies typically carry; it is not uncommon for flies to harbour upwards of 100 L3 larvae [53]. The emergence of larvae from the head causes great irritation to the fly, eliciting proboscis 'milking' actions associated with stress, and damage to the labio-hypopharyngeal membrane and musculature [54]. Indeed, whilst it has been shown experimentally that flies can remain infective for up to 5 days following the first L3 larval emergence, with infective larvae emerging each time a bloodmeal is taken over that period, the vast majority of flies die within 24 h of taking a bloodmeal in which L3 larvae emerge [16]. Hence, a single fly is very unlikely to transmit infection more than once during its lifetime.

Vector lifespan is of a duration comparable to that of the EIP of *L. loa*, and is significantly reduced by carriage of the parasite (independently of the high mortality rate associated with the emergence of infective L3 larvae; Figure 3). Earlier reviews have suggested that only 10%

belonging to the *Chrysops* genus, primarily *Chrysops silacea* and *Chrysops dimidiata*, during ingestion of a bloodmeal. Once ingested by the fly, and in a process taking 10–12 days, microfilariae develop into the third stage (L3) larvae, the stage capable of infecting people. This process is characterised by the transition between a number of discrete larval forms, with the microfilaria to L1 transition taking approximately 2 days, the L1 to L2 transition 4 days, and the L2 to L3, 4 days [100]. The L3 larvae reside primarily in either the abdomen or head, and upon initiation of a bloodmeal, are recruited from both sites, where they emerge and quickly escape into the cutaneous laceration inflicted by the fly in order to feed [52]. Once inside their human host, L3 larvae mature and moult, firstly into L4 larvae and then into juvenile worms (sometimes called L5 larvae) in a developmental processes taking 16–20 and 30 days, respectively [24]. The appearance of microfilariae in the bloodstream does not occur until much later, often as long as 150–170 days after infection [25], implying the existence of a further delay following development into a juvenile worm before adults become fully mature, capable of reproduction and production of microfilariae. Little is known about the lifespan of adult worms, although reports indicate that they can live for as long as 21 years [1].

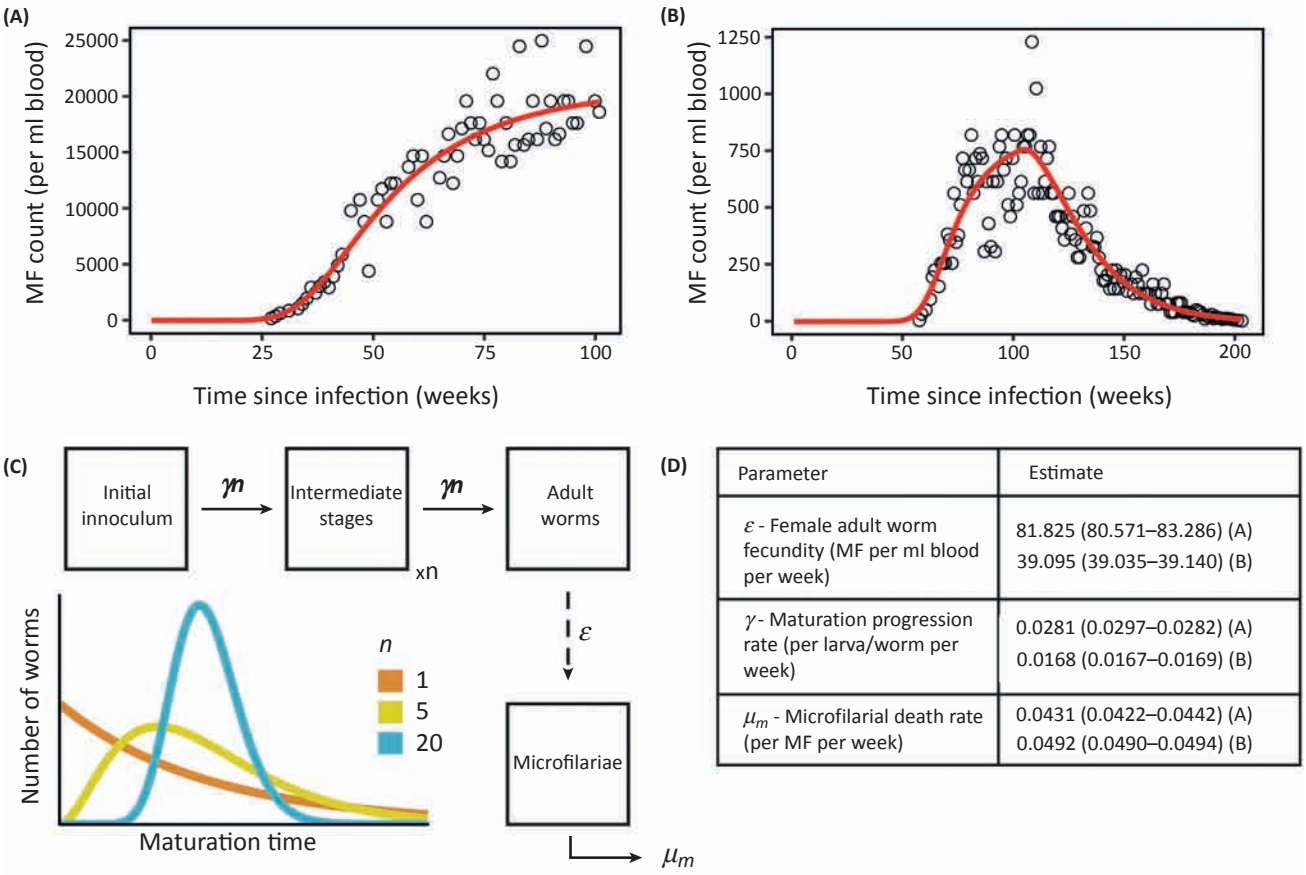

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Figure 2. Estimating Parasite Population Biology Parameters. Counts of blood microfilariae (MF) in a patas monkey (*Erythrocebus patas*) infected with 75 L3 larvae (panel A, open circles), and a baboon (*Papio anubis*) infected with one female and two male juvenile worms (panel B, open circles) were extracted from [25]. Mathematical models were fitted to these data (panels A and B, red line) using **maximum likelihood**. The specific pre-patent interval (time between infection and appearance of microfilariae in the peripheral blood) for each individual animal was not reported and so the average pre-patent period for each species (reported) was used in each case for purposes of calculating the pre-patent period. Both primates were splenectomised, and pre-splenectomy data were excluded due to the known involvement of the spleen in clearing infection in primates [11]. Data from experimental primate infections [26] were used to estimate key population biology parameters, namely average adult worm fecundity (ε), larval maturation (γ) and microfilarial mortality (μ_M) rates (panel D). The models fitted to the data are elaborations of the immigration-death framework [65] (panel C) incorporating a number of intermediate latent parasite stage compartments (n). This changes the distribution of maturation times or pre-patent periods without changing the mean of the distribution. For one compartment (the default for numerous population dynamics models), times are exponentially distributed. For increasing n, times are gamma distributed with shape factor given by integer n (strictly an Erlang distribution), leading to progressive narrowing and increased symmetry of the distribution. The best-fitting models included 22 and 75 compartments for the data from the patas monkey (panel A) and baboon (panel B), respectively, congruent with the inoculum used in each case (75 compartments yield a narrow distribution of maturation times approaching a point estimate, coherent with the fact the inoculum consisted of a single female worm). By contrast, 22 compartments result in a range of times, consistent with the patas monkey having been infected with a large number (75) of L3 larvae. To accommodate decreases in microfilarial concentration (panel B) (despite necropsy of the baboon yielding a living female worm), it was assumed that the inoculated males had ceased inseminating the female, or that senescence of the female had led to declining fecundity, which was modelled as exponentially declining over time; model fitting suggested fecundity to have begun declining at week 106, reaching 5% of its initial value by week 146. Little is known about the reproductive lifespan of *Loa loa*, but in *Onchocerca volvulus*, it is about 9–11 years, with simulated declines in fecundity starting from the patent age of 5 years and reaching 0 at age 20 [101], significantly longer than the times reported here, where decline starts at 2 years and is complete by 4 years. The differing fecundity, maturation, and microfilarial mortality rate estimates reported in panel (D) may reflect physiological differences between the two primate species because baboons are natural hosts for *L. loa*, whereas patas monkeys are not.

of flies survive the minimum 10 days required for microfilariae to develop into L3 larvae [3], and only 5% survive past the estimated 12-day upper bound of the EIP [41]. Whilst it is usually assumed that carriage of developing larvae incurs no excess mortality in the fly, our reanalysis of historical data shows a significant difference in survivorship between flies infected with high

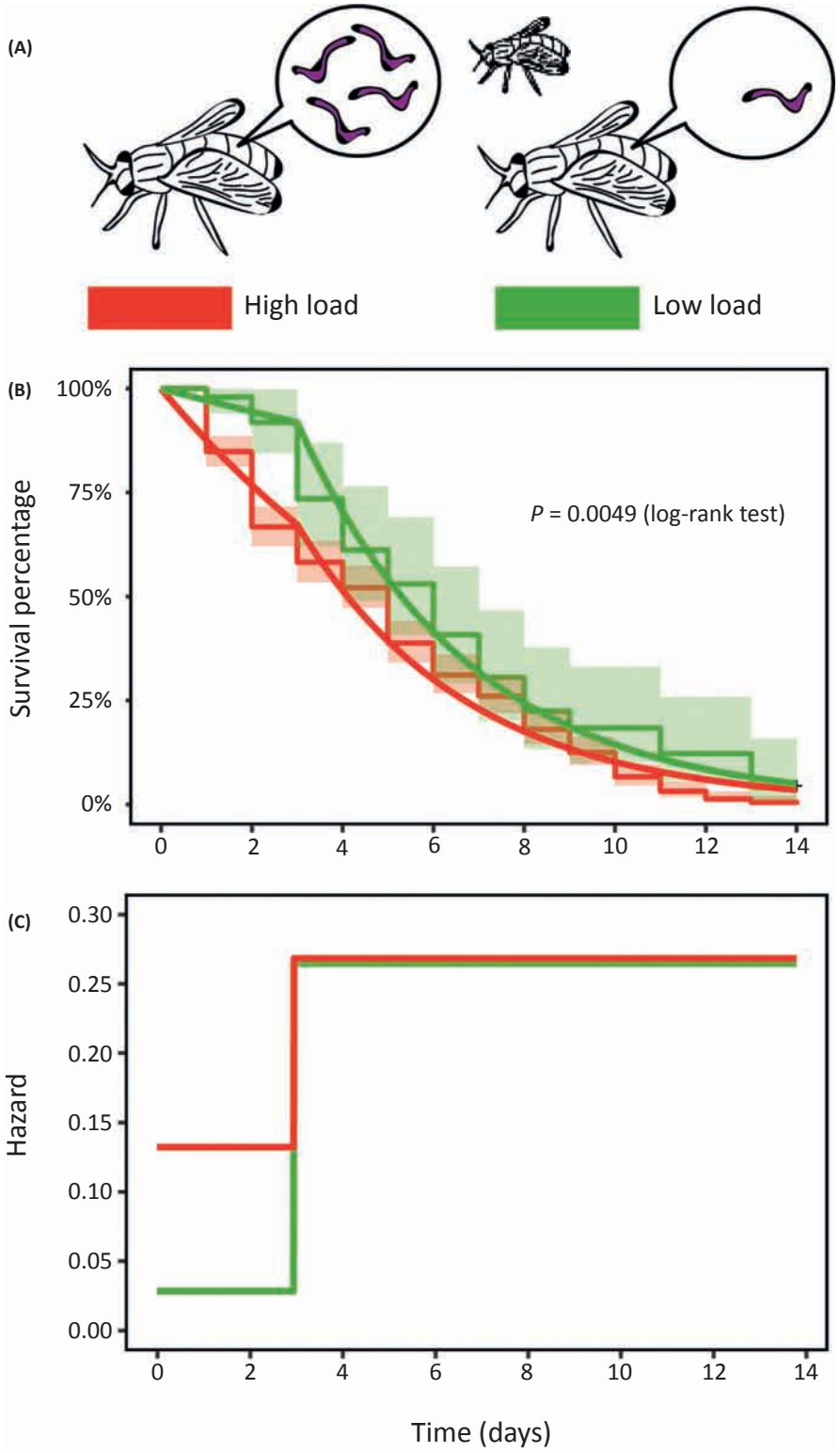

Figure 3. Estimating the Impact of Infection on Vector Mortality. Data from experimental feeding experiments were analysed to assess whether the ingested concentration of microfilariae from human blood affects tabanid *Chrysops*

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and low *L. loa* loads (Figure 3A,B). Fitting a piecewise exponential hazard function to the data highlights that much of the excess mortality arises from the ingestion of high numbers of microfilariae, occurring within the first 2–3 days following a bloodmeal (Figure 3C). This is similar to observations on excess mortality in blackflies ingesting *O. volvulus* microfilariae [54]. This conclusion is further supported by less formal evidence from other studies suggesting that infection with the parasite, and the intensity of the infection, both influence vector mortality [41,55].

Epidemiology of Loiasis

Geographical Distribution, Disease, and Host Species

L. loa is endemic across much of Central Africa, a region that is home to well over 30 million people [4], with areas within the DRC and Cameroon together accounting for almost 40% of the population at risk. An estimated 14 million individuals reside in high-risk areas, where the prevalence of eye worm passage (history of eye worm) is greater than 40% [4] (implying current or past infection with adult worms).

Clinical indicators of disease appear anywhere from 2 months [56] to 21 years [1] after initial infection. Most commonly observed symptoms include 'eye worm' and Calabar swellings [2], as well as a number of rarer, more severe pathologies, including renal, cardiac, and neurological involvement [3,57]. Besides this, treatment with ivermectin or **diethylcarbamazine (DEC)** of subjects presenting very high microfilarial densities can induce, within 1–3 days, a specific and potentially fatal encephalopathy.

In addition to infecting humans, several primate species (including the yellow baboon, *Papio cynocephalus* [58] and the mangabey, *Cercocebus albigena* [59], amongst others) have been found to be naturally infected with a strain of *Loa*. Although these parasites appear to belong to the same species that infects humans (as evidenced by their capacity to produce fecund hybrids with the human-infecting parasites [60]) the overall contribution of simian hosts to human loiasis is considered minimal due to the differing biting habits of their respective vectors (*C. silacea* and *C. dimidiata* for human *Loa*; *C. langi* and *C. centurionis* for the simian strain) and the differing periodicities of microfilarial circulation in the peripheral blood (adapted to the biting habits of their vectors – diurnal for human *Loa*, nocturnal for the simian form) [10,61].

species mortality and lifespan. Data were taken from [16] and comprise two groups of flies fed on people with either 'high' or 'low' concentrations of microfilariae in their blood (exact concentrations not given; panel A). Comparison of Kaplan–Meier survival curves for the two groups of flies (panel B) revealed a significant difference in survival ($P = 0.0049$, Mantel–Haenszel test). The main differentiating feature between the two curves occurs in the first 2 days, where flies feeding on humans with a high microfilarial concentration had greater mortality than flies feeding on humans with low concentrations. This is in keeping with observations on *Simulium* species of blackfly vectors of *Onchocerca volvulus*, in which ingestion of high microfilarial loads by the fly is associated with substantial excess mortality [54]. Motivated by this, a piecewise exponential model was fitted to the data (panel B), with different (constant) hazards over the initial 3 days but the same thereafter. Proportional hazards were assumed for the two groups of flies, and models were fitted using maximum likelihood techniques. The results confirmed a statistically significant 4.67-fold greater hazard for the first 3 days after ingestion of microfilariae in the flies feeding on humans with high concentrations compared to flies feeding on humans with low concentrations (panel C). By contrast, after the first 3 days, the difference in hazards between the two groups was negligible and not statistically significant. This suggest that higher intensity infections significantly impact vector mortality in a density-dependent manner. This conclusion is further supported by other research comparing flies fed on infected mandrills and uninfected rats which, although not producing a statistically significant difference, shows an appreciable difference between the mortality of infected and uninfected flies [41]. Other, older data also support this, with research by Kershaw *et al*. [55] suggesting that tabanid horseflies infected with *Loa loa* microfilariae have a lower life expectancy than their uninfected counterparts. It is, however, important to note that this same research also observed highly infected flies living longer than flies with low-intensity infections, although interpretation of these results is complicated greatly by the presence of *Mansonella perstans* co-infection and a small sample size compared to the data from Connal and Connal [16].

These factors are thought to contribute to the spatial and temporal separation of transmission between the two strains, although the possibility of some cross-transmission between host species should not be completely ruled out.

Prevalence and Intensity of Infection in Human Populations

A number of studies have observed that the community prevalence of *L. loa* microfilaraemia saturates between 50% and 60% (in the regions of highest endemicity) with increasing average microfilarial load (infection intensity; mean number of microfilariae per ml of blood) [62,63]. Prevalence and intensity remain fairly constant over time [64], indicating endemic stability and therefore the operation of density-dependent processes constraining the growth of the parasite population [65]. In onchocerciasis, density-dependent processes operate on microfilarial establishment within the (blackfly) vector [21], on (excess) vector mortality [54], and on adult worm establishment (with increasing annual transmission potential) in humans [66]. Research into the necessary processes that regulate *L. loa* populations is limited, but evidence from our own analysis of historical data (Figure 3), as well as more contemporary results (see below), supports the existence of excess vector mortality induced by ingestion of microfilariae. Whether other density-dependent processes operate in the *L. loa* life cycle remains unknown.

The shape of the loiasis prevalence–intensity relationship is superficially similar to that observed for other filarial infections such as onchocerciasis [67], as well as other helminth infections like schistosomiasis [68], except that in the latter two, the prevalence of infection with transmission stages (microfilariae or parasite eggs, respectively) can reach nearly 100% as infection intensity increases. This relationship has been explained by suggesting that the degree of parasite **overdispersion** among hosts (probably driven by heterogeneous exposure to infection) decreases as mean infection intensity rises, allowing for infection prevalence to reach high values (nearly 100%) when intensity is high. Interestingly, previous studies have suggested that the degree of *L. loa* overdispersion across communities is severe and does not change with mean infection intensity [69], an observation that could lead to the lower levels (50–60%) of saturating microfilarial prevalence that have been reported.

There are other notable differences in the epidemiology of loiasis compared with other helminthiases. The first is that the intensity of infection (microfilariae per ml of blood) has the capacity to far exceed that observed for other blood-dwelling filarial parasites. Very high microfilarial concentrations, reaching 50 000 per ml of blood, are not uncommon [70] and differ notably from values reported for LF, in which microfilarial concentrations in the blood rarely exceed 20 000 per mlAppendix A [71]. The second is that a high proportion of parasitised subjects (as determined by signs of apparent infection such as history of eyeworm), do not present with microfilaraemia (although a similar phenomenon is also observed in LF [72]). Indeed, despite high prevalence of apparent infection (history of eyeworm), which in hyperendemic regions can surpass 70% [4], with 95% of the population possessing antibodies reacting with *L. loa* antigens by the age of 2 years [73], the proportion of people with microfilaraemia (assessed by microscopy) is often far lower, typically representing 30–40% of the total population, and only 50–60% in highly endemic areas (and even then only in adults older than 50 years of age) [70]. It is often, therefore, concluded that a substantial fraction of the population is **amicrofilaraemic** [74].

Part of this disparity among current measures of detecting infections might be explained by the sensitivity of the diagnostics. The capacity of blood smears to accurately detect microfilariae in individuals with very low infection intensities is limited; resampling of individuals over time, studying larger volumes of blood than usually used in the thick smears used for microscopic

assessment, as well as using more sensitive PCR-based techniques, have shown that a significant proportion of apparently amicrofilaraemic individuals do in fact harbour microfilariae, albeit at levels below those detectable by microscopy [75–78]. The proportion of people who are actually microfilaraemic despite negative blood smears remains to be assessed, as does the reason behind the highly variable levels of microfilaraemia that can be seen in members of the same community (microfilarial concentrations ranging from 0 to more than 200 000 microfilariae per ml of blood, as quantified by microscopy [79,80]).

Heterogeneity in microfilarial concentrations could be caused by differences in exposure among individuals, innate differences in genetic traits [81] that mediate susceptibility to infection, immunological factors [82] that influence microfilarial dynamics in the body, or a combination of these mechanisms. The observed stability of microscopy-diagnosed microfilarial status (positive or negative) over significant time periods (in the order of years) in individuals living in endemic areas could be consistent with any of these explanations but does at least indicate that individuals maintain relatively stable (possibly very low) levels of infection (or remain consistently uninfected) throughout their lifetime [64,83,84].

Patterns of Microfilarial Infection by Human Age and Sex

In contrast with diseases caused by soil-transmitted helminths and schistosomes, whose worm burdens often peak in the young (excepting hookworm) [65], the age-specific patterns of *L. loa* microfilariae infection typically involve prevalence increasing monotonically with age [70,84,85] (Figure 4A). For schistosomiasis, the observation of a convex infection pattern is thought to be due to the slow development of acquired immunity to reinfection [86]. Such a peak is not observed for loiasis, suggesting that if there is a substantive role for protective immunity in shaping individual responses to loiasis, it is likely to be either innate or rapidly acquired, rather than gained gradually over many years of exposure to the parasite.

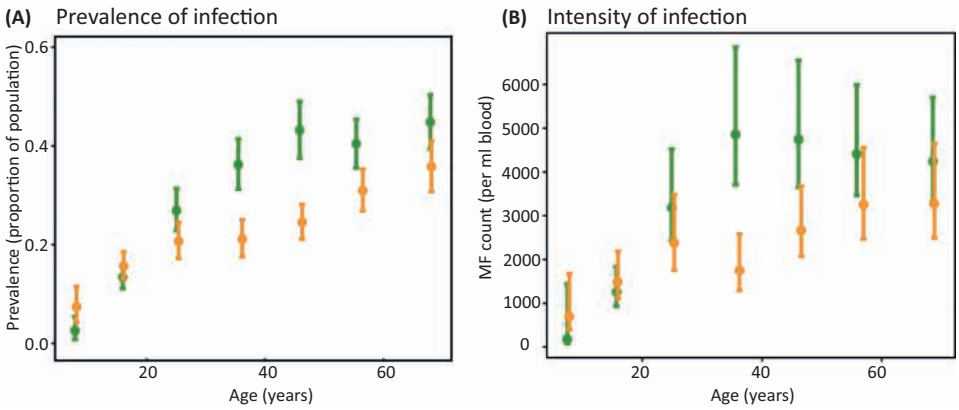

Figure 4. Patterns of *Loa loa* Infection by Human Host Age and Sex in the Administrative Division of Lekie in Central Cameroon. Markers represent the prevalence of microfilariae (MF) in blood (A) and the arithmetic mean number of microfilariae per ml of blood (B) in 10-year age groups plotted against the mean age within each group. Yellow markers refer to women, green markers to men. For prevalence estimates, confidence intervals were calculated using the Clopper–Pearson exact method [102]. For microfilarial infection intensity, the arithmetic means include both microfilariae-positive and -negative individuals (i.e., 0 and positive counts), and error bars represent 95% credible intervals for the mean of a zero-inflated negative binomial distribution fitted (using a Bayesian approach with uninformative/vague priors) to the individual data for each age-category and sex [103]. Data are from [62].

Like prevalence, mean intensity of microfilarial infection (averaging over both microfilaraemic and amicrofilaraemic individuals) also increases with age (Figure 4B), although intriguingly, after initial infection, an individual's intensity of infection (microfilarial concentration in the blood, as measured by microscopy) is relatively consistent over their lifetime. This has led researchers to suggest that infections with *L. loa* are non-cumulative [84], that is, observed increases in average microfilarial infection intensity (across a community, including both microfilariae-positive and -negative individuals) primarily reflect an increase in the number of individuals infected, rather than an increase in the parasite load of those already infected. This contrasts with many other helminthiases where prevalence saturates with increasing average intensity of infection, indicating that infection intensity is increasing in individuals already infected, but very few new individuals are becoming infected.

L. loa infection also shows sex-specific patterns (Figure 4), with a number of studies demonstrating that the prevalence of microfilarial infection is lower in females than in males across all ages [70,87]. It has been suggested that these observations may be explained by either occupationally mediated differences in exposure to infective tabanid vectors between men and women, or by physiological differences between the sexes. One study has shown that exposure is an important determinant of whether an individual is microfilaraemic or not [87], providing support for differential exposure being a potentially important driver of epidemiological patterns. However, these results contrast with those from an earlier study in which neither exposure nor sex were significantly associated with microfilarial status [84].

Concluding Remarks and Future Perspectives

Recent work reporting excess human mortality associated with loiasis [7], and the large numbers of people currently infected or at risk of infection [4] suggest that the notion of the disease as merely an impediment to the treatment of onchocerciasis and LF with ivermectin underestimates the true magnitude of the problem. Loiasis should be recognised as an NTD of significant public health importance worthy of attention and effort in its own right, and an assessment of its global disease burden should be undertaken (see Outstanding Questions). These initial steps would be vital to support optimal deployment of interventions to combat the disease, and help the millions at risk of loiasis in Central Africa who have been largely ignored.

Designing and implementing interventions against loiasis will require further research in different areas. These include better resolution of the spatial distribution of loiasis (particularly in intermediate- and low-transmission areas), something that may be facilitated by the recently developed antibody-detecting lateral-flow assay [88], as well as investigation of interventions other than ivermectin. Some work has already been carried out on this, including important exploration of vector control options [10], as well as investigations into albendazole as a treatment in instances where high blood microfilarial concentrations preclude treatment with ivermectin [89], but more research is needed.

Perhaps one of the most pertinent and timely research avenues would be the development of mathematical models of loiasis transmission and control. Given the often high levels of co-infection with onchocerciasis that exist in populations [79,90], the significant number of people with onchocerciasis who have already received ivermectin, and the commitment to ensure those still infected with *O. volvulus* receive the necessary treatment [91], it is likely that many individuals currently infected with *L. loa* will receive, or have already received, ivermectin. More recently, 'test and not treat' strategies have been proposed (and community trials implemented) in which individuals with a high infection level (>20 000–30 000 microfilariae per ml) are identified by rapid diagnostic tests (e.g., Cellscope Loa [92]) and not treated with ivermectin (the

Outstanding Questions

What is the global burden of disease due to morbidity and mortality associated with *L. loa* infection, and how could we best quantify its disability weights?

What will be the indirect impact of interventions targeting onchocerciasis and/or lymphatic filariasis on loiasis in the regions of Central Africa receiving regular preventive chemotherapy with anthelmintics such as ivermectin? Will such interventions be sufficient to eliminate loiasis in co-endemic areas, or will further loiasis-specific control measures be required?

In areas not co-endemic with onchocerciasis and/or lymphatic filariasis, what interventions would be most effective and cost-effective at interrupting loiasis transmission and eliminating the parasite, given its complex and incompletely understood population biology and epidemiology?

Are there any other density-dependent processes operating within the parasite's life cycle and further regulating its population abundance? If so, at what stage are they acting, and to what extent?

Is there a subset of the human population who is genuinely amicrofilaraemic, or is this observation due to the limited sensitivity of the currently available microscopy-based diagnostics?

Why is there such extreme variation in the levels of microfilarial infection among individuals? What are the relative roles of exposure, physiology, and genetics underlying this observation?

proportion of individuals who exhibit these heavy microfilaraemia levels typically represents 1–2% of the population) [92,93]. Without suitably constructed mathematical models, the impact of these strategies on the transmission dynamics of loiasis will remain unclear and amenable only to informal and less rigorous analysis. For example, it is known that community-directed treatment with ivermectin can effect significant reductions in *L. loa* prevalence, both in humans and in the *Chrysops* vectors [94,95]. But the impact of this intervention on loiasis transmission among diverse transmission settings, and the prospects of elimination, remain unclear. Construction of a mathematical model reflecting the epidemiological features of *L. loa*, both in the vector and human host, would therefore assist in assessing the indirect impact of interventions intended to control and eliminate onchocerciasis or LF and in evaluating the need for further interventions specifically targeting loiasis.

It would also further help to explore and understand the complex epidemiology of the disease; although loiasis shares similarities with other filarial infections, it is also marked by several distinct features that must be specifically incorporated into mathematical models to accurately reflect transmission dynamics, and in turn, whose incorporation will allow further exploration and analysis of their key properties. These include the distinct ecology of the tabanid *Chrysops* vectors (driven by an array of geographical and environmental factors [96,97] and with consequences for the parasite's transmission dynamics); the indicated role of individual- and age-specific exposure to infection; the postulated presence of an amicrofilaraemic subpopulation; the parasite's lack of *Wolbachia* endosymbionts precluding the use of antimicrobials as treatment (in contrast to onchocerciasis and LF against which they can be employed) [98]; and the extremely high microfilarial loads that individuals can sustain and that are associated with a risk of SAEs following treatment with microfilaricidal drugs. Indeed, in individuals with high microfilaraemia there are no safe and effective treatment options (although there are preliminary results suggesting that long courses of albendazole are safe and potentially effective [99]), and research into new antifilarial treatments is urgently needed. These features complicate the treatment and control of loiasis significantly, limiting the utility and safety of formulating intervention strategies without recourse to rigorous mathematical models of transmission dynamics. Construction of a mathematical model accurately reflecting the parasite's population biology and transmission dynamics will serve to increase our understanding of this disease and facilitate the implementation of effective measures to control or eliminate loiasis as a public health concern.

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Resources

[i]www.who.int/neglected_diseases/events/tenth_stag/en/

[ii]http://horizon.documentation.ird.fr/exl-doc/pleins_textes/pleins_textes_2/memoires/08233.pdf

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