REVIEW

Mansonellosis: current perspectives

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Abstract: Mansonellosis is a filarial disease caused by three species of filarial (nematode) parasites (*Mansonella perstans*, *Mansonella streptocerca*, and *Mansonella ozzardi*) that use humans as their main definitive hosts. These parasites are transmitted from person to person by bloodsucking females from two families of flies (Diptera). Biting midges (Ceratopogonidae) transmit all three species of *Mansonella*, but blackflies (Simuliidae) are also known to play a role in the transmission of *M. ozzardi* in parts of Latin America. *M. perstans* and *M. streptocerca* are endemic in western, eastern, and central Africa, and *M. perstans* is also present in the neotropical region from equatorial Brazil to the Caribbean coast. *M. ozzardi* has a patchy distribution in Latin America and the Caribbean. Mansonellosis infections are thought to have little pathogenicity and to be almost always asymptomatic, but occasionally causing itching, joint pains, enlarged lymph glands, and vague abdominal symptoms. In Brazil, *M. ozzardi* infections are also associated with corneal lesions. Diagnosis is usually performed by detecting microfilariae in peripheral blood or skin without any periodicity. There is no standard treatment at present for mansonellosis. The combination therapy of diethylcarbamazine plus mebendazole for *M. perstans* microfilaremia is presently one of the most widely used, but the use of ivermectin has also been proven to be very effective against microfilariae. Recently, doxycycline has shown excellent efficacy and safety when used as an antimicrobial against endosymbiotic *Wolbachia* bacteria harbored by some strains of *M. perstans* and *M. ozzardi*. Diethylcarbamazine and ivermectin have been used effectively to treat *M. streptocerca* infection. There are at present no estimates of the disease burden caused by mansonellosis, and thus its importance to many global health professionals and policy makers is presently limited to how it can interfere with diagnostic tools used in modern filarial disease control and elimination programs aimed at other species of filariae.

Keywords: mansonellosis, filariasis, *M. perstans*, *M. ozzardi*, *M. streptocerca*, neglected disease

Introduction

Mansonellosis is a filarial disease caused by various species of the genus *Mansonella* (Nematoda; Filarioidea; Onchocercidae) that use humans as their primary definitive hosts.[1] By most modern definitions, there are just three filarial species considered responsible for causing human mansonellosis: *Mansonella perstans*, *Mansonella streptocerca*, and *Mansonella ozzardi*.[1–5] Other *Mansonella* spp., like the chimpanzee parasite *Mansonella rodhaini*, occasionally infect humans, and some can use humans as definitive hosts,[6–9] producing patent infections with circulating microfilariae. However, they are rare infections in humans in comparison with other primates, and by convention they are not considered to cause mansonellosis.[1–5]

As *M. ozzardi* was originally the only parasite of the *Mansonella* genus known to infect humans,[10] the term "mansonellosis" and its synonym "mansonelliasis" were, until the mid-1980s, applied only to infections caused by this one species.[11–15] The

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modern use of the term[1–5] began only after Orihel and Eberhard revised the genus *Mansonella* and added *M. perstans* and *M. streptocerca*.[16,17] Prior to this, a number of systems of nomenclature were used to describe *Mansonella* parasite infections,[18–24] eg, mansonellosis was usually restricted to infections caused by *M. ozzardi*[19,20] and the terms "perstans filariasis" and "streptocerciasis" were often used to describe infections with *M. perstans* and *M. streptocerca*, respectively.[18,21–24]

Among the known human filarial infections, mansonellosis is probably the most prevalent[23,25] but nevertheless the least studied, and can be considered the most neglected filariasis. Certainly, it is more prevalent and more neglected than other filarial diseases like lymphatic filariasis, onchocerciasis, and loiasis. It can even probably be considered one of the most neglected of all the tropical diseases, as it is subject to more neglect than schistosomiasis, taeniasis, echinococcosis, or rabies. [1–5]

The three species of *Mansonella* parasite which cause mansonellosis in humans vary in biological, clinical, and epidemiological characteristics (Table 1).[1–5,18,23–25] The life cycles for all three species are similar, involving development in two hosts: an insect and a primate.[4,15,25–27] Humans are the only known natural vertebrate host for *M. ozzardi*, but other primates can serve as natural hosts for *M. perstans* and *M. streptocerca*.[2,28] All of the known insect vectors of mansonellosis are from the Diptera taxonomic grouping. The known vectors of *M. perstans* and *M. streptocerca* are from the biting-midge genus *Culicoides*. *M. ozzardi*, however, is not exclusively transmitted by *Culicoides*.[15,26,27,29] A range of non-*Culicoides* Ceratopogonidae biting midges and blackflies from the genus *Simulium* are known to be capable of transmitting *M. ozzardi* (Table 1).[15,26,27,29]

Females of all known vectors of mansonellosis consume blood meals in order for their eggs to mature.[26,27] Microfilariae are ingested by the vector when a female vector takes a blood meal from an infected host. The microfilariae penetrate the insect's gut and go through several maturation stages in the thoracic flight muscles over 6–12 days before migrating to the head and proboscis, from where they can be transferred to a primate when the vector takes its next blood meal.[4,26,27] During the blood meal, the third-stage (infective) filarial larvae actively break out of the mouthparts and penetrate into skin via the bite wound before migrating and maturing into adult worms. Males and females mate, and female worms begin to produce unsheathed microfilariae that circulate in the blood (*M. perstans* and *M. ozzardi*) or diffuse into the skin (*M. streptocerca* and *M. ozzardi*) of the primate host.[4,26,27] Practical diagnostic guides often describe all three species

as having aperiodic microfilariae that circulate in peripheral blood or skin throughout the day and night,[30] although reports of cryptic periodicity for both *M. perstans* and *M. ozzardi* exist.[4,31,32]

Most of what is known of mansonellosis and the geographic distribution of *Mansonella* parasites is based on the morphological identification of microfilariae recovered from blood or skin-snip samples.[23] With adult filarial parasites, almost impossible to recover, even accounts of "new species" of *Mansonella* or *Mansonella*-like parasites have often been based solely on the morphological characteristics of microfilariae.[33–36] The species-diagnostic characters of microfilariae are sometimes difficult to see, and can vary greatly with sample preservation, mounting, and staining procedures,[4–38] and hence microfilariae are easily misidentified.[39,40] This has resulted in a number of questionable "new" *Mansonella* species being described,[33–36] some controversial filarial parasite-distribution maps (generated by *Mansonella* parasites being confused with other filarial parasites and vice versa),[39,40] inappropriate clinical treatments,[39] and even dubious clinical symptoms being attributed to mansonellosis infections.[41] For example, there has only ever been one very tentative report of *M. perstans* encountered in the cerebrospinal fluid of patients. The report concerned patients from the Zambezi valley region of Zimbabwe,[41] and has subsequently been attributed to a zoonotic filariasis,[7,42,43] but there are a number of published papers where *M. perstans* is described as occasionally causing severe neurological symptoms.[15,44–47]

Following the development of filarial parasite-diagnostic polymerase chain reaction (PCR) assays, many ambiguities concerning species' distribution and "new species" have been resolved, and most modern filarial parasite research, including mansonellosis research, now suffers from far fewer parasite-identification uncertainties than used to be the case.[48–53] Almost all of these assays use mitochondrial or rDNA target sequences, and were developed from molecular systematic studies (see "Diagnosis" section), which used DNA sequences from these regions to constructed molecular filarial parasite phylogenies.[48–57] Although these mitochondrial and rDNA phylogenetic studies and more recent multilocus sequence typing challenged traditional morphology-based phylogenies of the species within the family Onchocercidae,[58] none has hitherto challenged the morphology-based genus *Mansonella* proposed by Eberhard and Orihel in 1984, from which the modern use of the term "mansonellosis" has arisen.[16,17,50,54–60]

Later molecular phylogenies investigated the origins of the filarial endosymbiont *Wolbachia*, which infects many

Table 1 Main characteristics *of Mansonella perstans, Mansonella ozzardi*, and *Mansonella streptocerca*

		M. perstans	*M. streptocerca*	*M. ozzardi*
Microfilaria tail		Blunt, rounded tail, body Nuclei extend to tip of tail	Hooked shape Body nuclei extend to tip of tail	Long, thin, pointed tail Body nuclei do not extend to tip of tail
Sheath in microfilariae		Unsheathed	Unsheathed	Unsheathed
Microfilariae: periodicity and localization		Aperiodic microfilariae Blood	Aperiodic microfilariae Skin of the upper trunk and shoulder girdle	Non-periodic microfilariae Blood and skin
Microfilaria: length × diameter		200×4–5 mm	180–240×3–5 mm	163–203×3–5 mm
Adults measurements		♂ 3.5–4.5 cm, ♀ 5–8 cm	♂ 1.3–1.8 cm, ♀ 2.7 cm	♂ 2.4–2.8 cm, ♀ 3.2–8.1 cm
Adults localization		Serous body cavities, mainly peritoneal, but may also appear subcutaneously	Subcutaneous tissues of the dermis	Thoracic cavity and mesenteries of the peritoneal cavity
Vector	Africa	*Culicoides* spp. (biting midges)	*Culicoides* spp.(biting midges)	
	Central America			• *Simulium sanguineum* (Panama)
	South America	*Culicoides* spp. (biting midges)		**Simuliidae** • *Simulium exiguum* (Argentina); *S. amazonicum* (Venezuela, Colombia, and Brazilian Amazon); *S. argentiscutum* (Colombia and Brazilian Amazon); *S. oyapockense* (Colombia, Venezuela, and Brazil); *S. sanguineum* (Colombia); *S. guyanensis* and *S. sanchezi* (Venezuela); *S. minusculum* (Guyana) **Ceratopogonidae** • *Culicoides insinuatus* (Colombia); *C. guttatus* (Guyana, Suriname and Venezuela); *C. paraensis* (French Guiana, Argentina and Bolivia); *C. debilipalpis* (Bolivia and Argentina); *C. lahillei* (Argentina)
	Caribbean			• *C. furens* (Haiti and St Vincent); *C. barbosai* (Haiti); *C. paraensis?* (St Vincent); *C. phlebotomus* (Trinidad); *Leptoconops bequaerti* (Haiti)
Geographical distribution		Central and west Africa, South America	Central and west Africa, western Uganda	Latin America and Caribbean islands
Reported clinical symptoms		Often asymptomatic Itching, pruritus, joint pains, enlarged lymph glands, Calabar swelling, neurological manifestations	Often asymptomatic Dermatitis, pruritus, rash, papular skin, inguinal adenopathy, occasional dizziness	Often asymptomatic Moderate fever, headache, articular pain, rash, sensation of coldness in legs, foot edema and face edema, keratitis, or corneal opacity
Treatment		Diethylcarbamazine + mebendazole: 200 mg/12 hours + 100–200 mg/day × 21 days Mebendazole: 100 mg/12 hours × 30 days Doxycycline: 200 mg/day × 6 weeks	Diethylcarbamazine: 6 mg/kg/day × 12 days Ivermectin: 150 µg/kg, single dose; not active against the adult worm	Ivermectin: 200 µg/kg, single dose Not active against the adult worm

(though not all) filarial parasites.[61–64] Data from these studies suggested that filarial parasites had acquired *Wolbachia* endosymbionts several times independently during the evolution of Onchocercidae.[62–64] These *Wolbachia* endosymbionts have important roles in the pathology of onchocerciasis and lymphatic filariasis, and are also necessary for the reproduction and development of filarial parasites that cause these diseases. This has made them an important target for therapeutics.[65]

The *Wolbachia* bacteria that infect *M. ozzardi* and *M. perstans* appear to share a common origin, but are genetically very distinct (belonging to the F superclade of *Wolbachia*) from the *Wolbachia* known to cause disease pathologies.[64] It is presently not clear if what is known about other filarial *Wolbachia* can be safely extrapolated to the *Mansonella Wolbachia* endosymbionts and if they have a hitherto-uncharacterized role in mansonellosis pathology or indeed what their role in *Mansonella*-parasite development and reproduction is.[65,66] However, in one clinical trial in Mali, antibiotic treatment with doxycycline was seen to provide sustained clearance of *M. perstans* parasitemias 12 months following treatment, suggesting that *Wolbachia* may have an important role in the reproduction and survival of adult worms.[67]

Wolbachia from *M. perstans* cannot be easily detected, and some *M. perstans* parasites may not be infected.[66–69] It has been proposed that *Mansonella* parasites may have acquired their F-clade *Wolbachia* (the only *Wolbachia* superclade found in both arthropod and filarial worm hosts) more recently than other filariae, and it may not be an essential symbiont for some *Mansonella* spp. and strains.[66] Consistent with this is the notion that *M. perstans* may be a species complex, and a potential new species or subspecies of *M. perstans* was recently described from rDNA ITS1 sequences. This potential "new species" was recovered from human blood samples taken in Gabon, and has provisionally been called *Mansonella* sp. "Deux".[60] A phylogenetic tree, constructed with Treecon software[70] by the neighbor-joining method, shows that *Mansonella* sp. Deux forms a sister clade to standard forms of *M. perstans* from Brazil and Africa (Figure 1). However, further studies should be performed to characterize this new species and evaluate its clinical significance in humans.[60]

## Epidemiology and distribution

Mansonella parasites are among the most common cause of blood parasitemias and are encountered widely across Africa and Latin America.[3,4,18,23,25,71] *M. perstans* is considered the most common of the mansonellosis parasites and is endemic in a large portion of sub-Saharan Africa, as well as a northern part of the Amazon rainforest stretching from equatorial Brazil to the Caribbean coast of South America, with molecular analysis suggesting that the parasite arrived in South America relatively recently and most likely as a consequence of the slave trade (Figure 2).[18,23,25,50] From the mid-1920s until the early 1970s, *M. perstans* was frequently recorded as occurring in Papua New Guinea, but there is very little primary-source data to support this and no recent survey
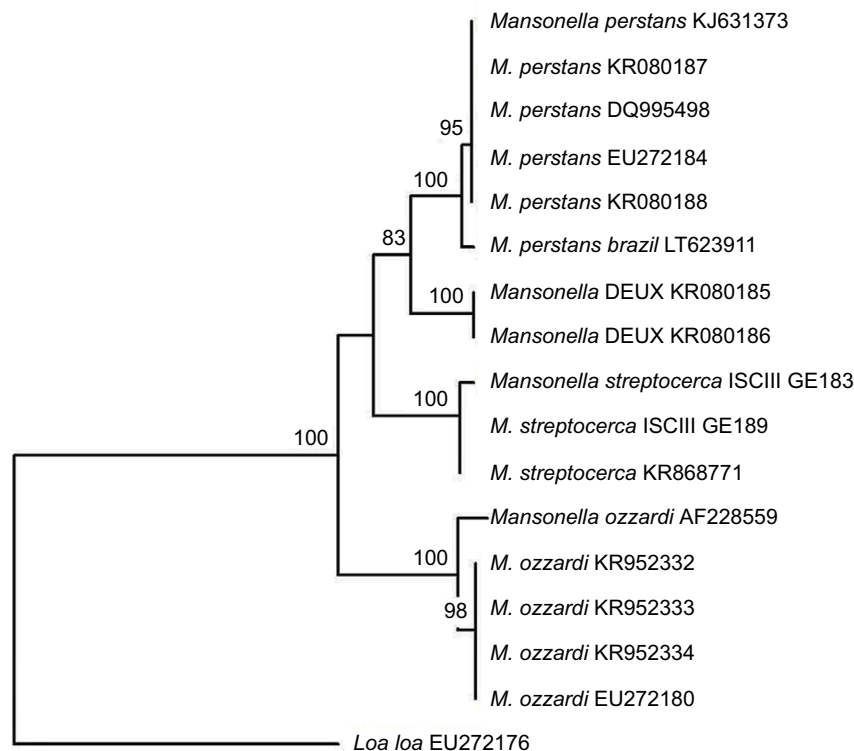
Figure 1 Neighbor-joining phylogenetic tree.
Notes: Tree compares the ITS1 partial sequences of human *Mansonella* spp. present in the GenBank (accession numbers are indicated in parentheses or with an internal code when not submitted to the GenBank). A sequence of *L. loa* (EU272176) was used as the out-group. Significant bootstrap values are indicated.
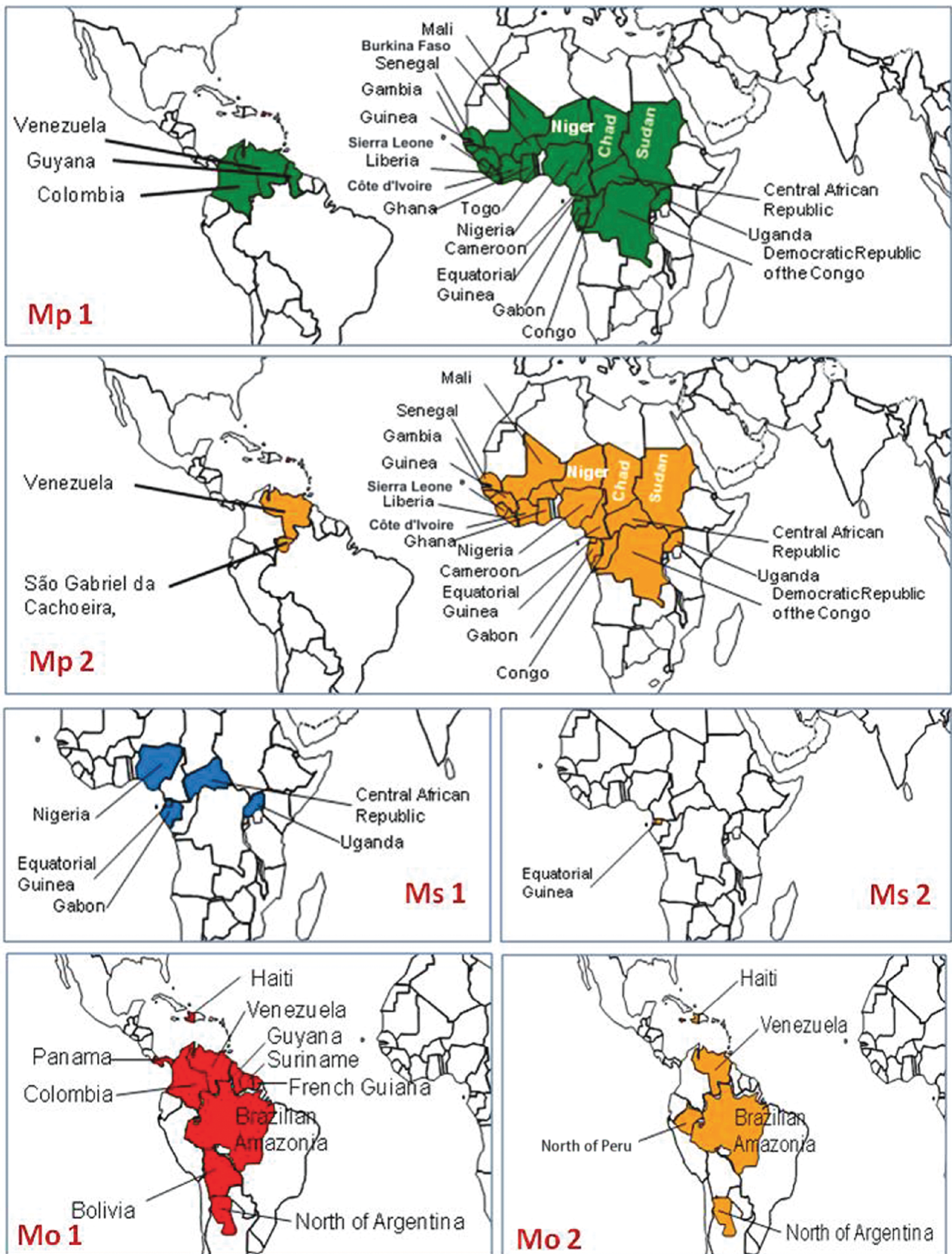
Figure 2 Distribution of human *Mansonella* based on published reports before and after the year 2000.
Notes: I, Before; 2, after.
Abbreviations: Mp, *Mansonella perstans*; Ms, *Mansonella streptocerca*; Mo, *Mansonella ozzardi*.

data that indicate its occurrence anywhere in the Eastern hemisphere outside continental Africa.[71] Overall, more than 100 million people may be infected by *M. perstans*, and it is estimated that 600 million people live at high risk of contracting an infection in Africa alone.[23]

A strong epidemiological link between banana plantations and *M. perstans* prevalence and parasitic loads was recorded more than 100 years ago, before its *Culicoides* vectors had been incriminated in its transmission (Table 1).[4,32,72,73] Sharp (the man who incriminated *Culicoides* in the transmission of *M. perstans*) was aware of this epidemiological link and presumably also that *C. austeni* uses banana-tree stumps and litter to support its larval development (because this had been published in the original description of the *C. austeni*). It is not clear to what extent, if any, the banana-plantation epidemiological observation led to elucidation of the life cycle of *M. perstans*.[32] Certainly, the epidemiological *M. perstans* studies that have been carried out since then have not provided much more significant understanding of the parasites' biology and transmission dynamics. However, they have shown that in endemic areas, prevalence of *M. perstans* infection is higher among older age-groups and that men are at more risk of infection than women.[23–25] Recently, geographic information-system data obtained from remote satellite imaging have been used in conjunction with *M. perstans* prevalence from school surveys to show *M. perstans* distribution varies with diurnal temperature range, vegetation, and cattle density.[74] Epidemiological studies that have tried to link *M. perstans* parasitemias with clinical symptoms have thus far failed to detect any significant correlations.[23,24]

M. streptocerca seems to have a distribution limited to continental Africa, where it occurs in the tropical rainforest areas of central and west Africa, as well as in Uganda (Figure 2).[4,18,25] Perhaps because the skin-snip biopsies required for its epidemiological monitoring are more invasive and painful than the blood sampling required for *M. perstans* surveys, or perhaps because blood sampling is simply more commonly done for other epidemiological studies, there have been fewer epidemiological surveys investigating *M. streptocerca* epidemiology.[4,25] As with *M. perstans* and *M. ozzardi* and many other filarial infections, prevalence rates of *M. streptocerca* are highest in the oldest age-groups studied and higher among men than women in endemic areas.[21,25,75] *M. streptocerca* is transmitted by various species of *Culicoides* and some of the same vector species that transmit *M. perstans*, although there are too few data available to evaluate properly whether its distribution is influenced by the same ecological drivers as *M.* perstans (Table 1).[15,77,78] The few

epidemiological studies that have tried to link patent *M. streptocerca* infections with clinical symptoms have encountered similar rates of skin disease among those infected and those uninfected in their study areas.[75,76]

M. ozzardi has a patchy geographic distribution across Latin America. It has been recorded from southern Mexico to northwestern Argentina, but has not been reported in Chile, Uruguay, or Paraguay.[3,4,18,25,29] The parasite occurs on several Caribbean Islands, and like *M. perstans* has even been recorded in Papua New Guinea, although recent reports of its occurrence in the region are notable by their absence (Figure 2).[4,71] In the Caribbean region, the parasite is transmitted by a diverse range of biting midges from the family Ceratopogonidae, whereas in Central and South America it is known only to be transmitted by biting midges from the genus *Culicoides* and blackflies from the genus *Simulium*.[3,4,15,29,79–90] Because of these vectorial differences, it was once thought that *M. ozzardi* from the Caribbean islands and those from continental South America might be different species (Table 1).[85,91] Artificial-infection experiments and the recent publication of DNA sequences, however, strongly indicate that parasites from the two regions are morphologically, biologically, and genetically near identical, and thus that they are most parsimoniously regarded as just one species.[2,17,28,48,50,58]

Even though the various populations of *M. ozzardi* are best considered as belonging to the same species, it is not safe to assume, for example, that the epidemiology of *M. ozzardi* in the Brazilian Amazon region is more or less identical to its epidemiology in the Caribbean, because of the great diversity of vectors involved.[3,4,15,29,79–90] In the Amazon region, although it is transmitted by a range of *Simulium* and *Culicoides* species, the parasite appears to be most commonly transmitted by *Simulium* vectors from the *amazonicum* species group and to have a distribution that follows the riverine breeding sites of these *Simulium* vectors.[29,79] In Trinidad, by way of contrast, the only known vector is *Culicoides phlebotomus* (which uses sandy-beach breeding sites), and the parasite thus has a coastal distribution.[15,88]

However, it is important to note that vector distribution is not the only important driver of *M. ozzardi* distribution. For example, repeated *M. ozzardi* surveys in the Brazilian Amazonian state of Rondônia have found the area completely devoid of the parasite, despite the fact that the region shares a similar ecology and blackfly fauna to the neighboring Amazonas state. It has been proposed that low levels of human immigration may play an important role in the parasite's failure to establish in the region.[91] However, data from tourists indicate that *M. ozzardi* infections are easily acquired, and as Rondônia is

surrounded by highly endemic areas, migration levels would have to be very low indeed for this to be the most important factor limiting the parasite's establishment in the region.[19,91–95] Furthermore, since the first discovery of *M. ozzardi*, surveys have shown that very small isolated foci (in some cases, the size of a single village) on numerous islands can sustain themselves over prolonged periods, suggesting that even low levels of migration could supply a sufficient parasite reservoir to allow for the establishment of a new focus if the other epidemiological criteria for establishment were met.[96] In Cameroon, it has been proposed that some areas of low onchocerciasis endemicity are explained by "zooprophylaxis", whereby exposure to simuliid bites from insects infected with cattle parasite *Onchocerca ochengi* provide something akin to natural vaccination.[97,98] In light of these observations, the recent discovery of highly prevalent zoonotic *Mansonella* parasites in anthropophilic simuliid vectors of the region may thus been seen as an alternative explanation as to why *M. ozzardi* has failed to establish in Rondônia.[94,98,99]

In line with epidemiological studies of other mansonellosis, research in the Brazilian Amazon has identified outdoor workers, males, and older community members as being at elevated risk of developing *M. ozzardi* parasitemias.[101,102] Importantly too, *M. ozzardi* parasitemias from this region have been shown to be positively correlated with joint pain, leg chills, headaches, and corneal lesions.[103,104]

Pathology and symptomatology of mansonellosis

Although mansonellosis has very broad global distribution and is almost certainly the most common form of filarial parasite infection, little is known about its pathology or symptomatology, with most health experts and policy makers viewing the parasites as largely innocuous and only rarely causing mild clinical symptoms.[4,5,18,25] However, as mentioned, recent studies investigating *M. ozzardi* infections in the Brazilian Amazon have found significant correlations between *M. ozzardi* infections and certain clinical symptoms, and other similar epidemiological studies investigating *M. ozzardi* in other regions and *M. perstans* and *M. streptocerca* have repeatedly failed to correlate clinical symptoms robustly with patent mansonellosis infections.[101–107]

This of course does not mean that mansonellosis is necessarily always completely asymptomatic outside the Brazilian Amazon region. Most of the epidemiological studies used to investigate symptomatology have had very small sample sizes and used diagnostic techniques of low sensitivity (ie, light microscopy-based diagnosis of thick blood smears), and

thus could not be expected to have had sufficient statistical power to detect subtle or complex correlations.[24,75,76,101–109] Recent observations suggest that mansonellosis infections can influence the human immune system's response and this can influence the development of secondary infections, like malaria.[110] As mansonellosis infections are chronic and occur in many tropical areas where a great number of other infectious diseases also exist, it is possible that the way mansonellosis influences other pathogenic infections could actually account for a very substantial proportion of its pathogenicity and its total disease burden.[4,5,18,23,25] At present, however, although there have been many accounts of mansonellosis parasites co-occurring with other parasites, there have been very few accounts about how mansonellosis influences the pathogenicity of other infectious diseases.[96,110–114]

Most symptoms ascribed to *M. perstans* infections in modern scientific literature are based on symptoms that have been recorded in case study reports.[23,25,44,115,116] As most of these reports are based on the treatment of tourists and expatriate Europeans and North Americans returning home from endemic areas, and not on people who have lived all their lives in endemic areas, it is not clear whether the symptoms reported from these studies can be used to compile a clinical picture that represents all or even most infections caused by mansonellosis.[23,25,44,116,117] Based on these reports, *M. perstans* can be considered to have little pathogenicity and almost always to be asymptomatic, but it can occasionally cause itching, joint pains, enlarged lymph glands, and vague abdominal symptoms.[5,23,25,116,117]

High-level eosinophilia are regularly observed in some but not all patients with *M. perstans* infections, probably as a consequence of the body's reaction against the adult worm, rather than against microfilariae.[118] *M. perstans* is also reported to be able to induce dermatological symptoms like the Calabar swellings of loiasis, fever, headaches, and pain in bursae and/or joint synovia or in serous cavities.[23,25,44,118] There has also been ocular pathology ascribed to *M. perstans* in which yellowish nodules develop in the bulbar conjunctiva; it has been reported that this pathology can on occasion provoke edema of the eyelids and proptosis.[7,118] This pathology, which has been described as a rare clinical manifestation of *M. perstans* infections caused by adult *M. perstans* migrating to the eye, is commonly referred to as "bulge" or "bung" eye disease.[7,118] Whether, however, this pathology is caused by *M. perstans* or a zoonotic parasite from the *Mansonella* genus is not presently clear.[7,118] Certainly, it is not uncommon for zoonotic filarial infections to have ocular clinical manifestations of this type.[7,8]

In addition to showing that *M. ozzardi* infections are associated with joint pain, leg chills, headaches, and corneal lesions, epidemiological studies have also added support to the notion that *M. ozzardi* infections are mostly asymptomatic.[101–105] Case reports from tourist-acquired infections have also suggested that *M. ozzardi* infections can cause fatigue, respiratory problems, dermal swellings, and itching; however, evidence that such symptoms occur regularly in endemic settings is still lacking.[19,95]

There have been very few studies of *M. streptocerca* infections. From what data are available, however, it is clear that infections with this parasite are very often (if not exclusively) asymptomatic.[4,5,21,22,25,75] However, it has been proposed that infections cause various types of dermatological symptoms in the shoulders and thorax, where the parasite is mostly encountered.[4,5,21,22,25,75] Hyperpigmented macules have been attributed to infections occurring in the Democratic Republic of the Congo, but have not been reported everywhere the parasite is found, including Uganda, and thus some authors have cautioned against their attribution to *M. streptocerca* infections.[21,22,75] Chronic papular dermatitis has been encountered in most *M. streptocerca*-endemic areas and has been reported to be cleared from patients successfully treated for *M. streptocerca* infections.[75] However, in endemic areas where epidemiological studies have been performed, chronic papular dermatitis is no more common in those patients who have detectable *M. streptocerca* in their skin snips than in those that do not, and thus at present there is no statistically robust evidence to support the parasites causing any symptoms at all.[4,5,21,22,25,75]

Diagnosis
Clinical diagnosis

As indicated in the previous section, very few clinical symptoms have been robustly linked to mansonellosis in general,[4,5,25] and at present there is robust evidence to support the notion that only *M. ozzardi* infections cause any clinical symptoms at all.[103,104] Therefore, it is near impossible to make a reliable clinical diagnosis of mansonellosis, even for *M. ozzardi*-caused mansonellosis. This is because most of the symptoms that have statistically robust associations with mansonellosis, such as joint pain, are so aspecific, difficult to score, and caused by numerous other pathogens that occur in mansonellosis endemic region as to be of almost no utility at all.[101–103] Even the potential of corneal lesions for clinical diagnosis is limited, because although they are quite a perceptible physical symptom, they are quite aspecific, occurring in both infected and uninfected individuals from

M. ozzardi-endemic areas. Furthermore, corneal lesions are not manifest in everyone who is infected or even occur everywhere that *M. ozzardi* occurs (eg, there are no reports of *M. ozzardi* causing corneal lesions anywhere in the Caribbean area).[103,104] In summary, it is presently impossible to use the clinical presentation of mansonellosis to make a reliable diagnosis of the disease.

Parasitological diagnosis

Traditional parasitological diagnosis, which is based on direct observation of a causative parasite, is still the most common way mansonellosis infections are diagnosed. It is performed by the detection and identification of sheathless *Mansonella* microfilariae in the skin or blood at any time of day or night.[4,5,18,25,30] Detection of skin-dwelling microfilariae is usually done by examination of ~1.5 mg (~2 mm diameter) skin-biopsy (skin-snip) samples, taken with a Walser or Holth corneoscleral punch.[4,5,18,25,30,119] The skin-snip test requires that a skin biopsy be taken from areas of presumed optimal microfilariae density, and this varies depending on geographic location. Blood samples used for mansonellosis diagnosis are often taken from peripheral blood samples by way of finger pricks, although venous blood samples can also be used for this purpose.[4,5,18,25,30,119]

When microfilariae are present in high numbers in the patient's blood, as is often the case in endemic areas, they may be easily found and identified in thick or thin blood smears stained with Giemsa or hematoxylin.[5,25,30,119] Skin snips are usually incubated in water or saline, and the emergent microfilariae can be stained on microscope slides, but in fresh unstained wet preparations of skin snips, the way in which the microfilariae move can also help with their identification. The main diagnostic microfilariae characteristics can be found in Table 1.[25,29,30,38,119] Morphologically, the most important features to identify microfilariae in blood smears stained with Giemsa are size and shape of the tail, presence or absence of a sheath, and the arrangement of terminal nuclei (in the tail).[18,25,30,38,119] All *Mansonella* microfilariae are unsheathed, and for diagnostic purposes are treated as aperiodic.[5,18,25,30,38,120]

The microfilariae of *M. perstans* are reported to be 200×4–5 µm, to have blunt rounded tails, and to have nuclei extending to the tips of their tails (Figure 3). *M. perstans* is easily distinguishable from other blood-dwelling microfilariae, which have overlapping distributions (*Loa loa* or *Wuchereria bancrofti*), by their being smaller, their lack of an enveloping sheath, and their tail features (their terminal nuclei are bigger than the other microfilariae).[5,18,25,30,119]
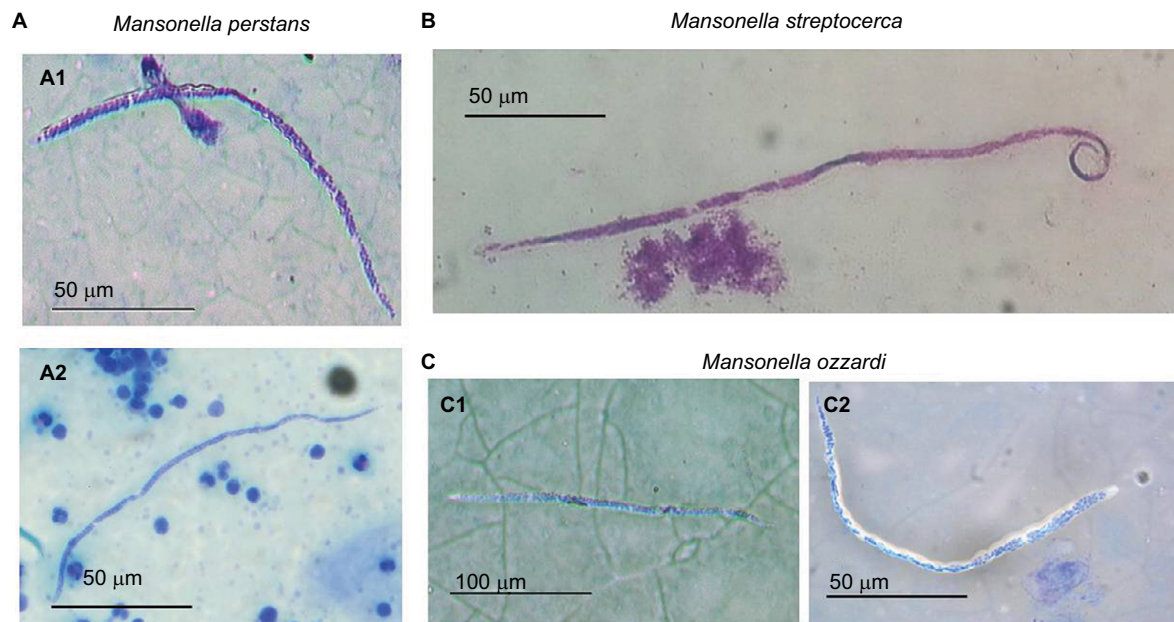
Figure 3 Comparison of the three agents causing mansonellosis illustrating the morphological characteristic of each microfilariae.
Notes: (**A**) *M. perstans* microfilariae seen in Giemsa-stained thick blood smear with blunt rounded tail and nuclei extending to the tip of the tail: *M. perstans* parasite from São Gabriel da Cachoeira (Amazonas state, Brazil) (A1); African *M. perstans* (A2). (**B**) *M. streptocerca* microfilariae from a skin-snip fixed and Giemsa-stained showing the characteristic hooked tail. (**C**) *M. ozzardi* microfilariae from São Gabriel da Cachoeira (Amazonas state, Brazil) stained with Giemsa (C1, C2).

M. streptocerca microfilariae are smaller and thinner (180–240×3–5 µm) than *Onchocerca volvulus*, and have hook-shaped tails with nuclei that extend to the end (no nuclei are seen in the pointed tail region of *O. volvulus* microfilariae) (Figure 3).[5,18,25,30,119] In wet mounts, live *M. streptocerca* microfilariae are usually less motile than those of *O. volvulus*.[5,119] With these morphological descriptions, the two filariae should be easily distinguished. In the past, however, in some countries, such as Uganda, *M. streptocerca* and *O. volvulus* have been confused, despite the clear morphological differences between them.[30,39,75]

The microfilariae of *M. ozzardi* are reported to be 163–203×3–5 µm, with long thin pointed tails and body nuclei that do not extend to the tips of their tails.[2–5,30,38] As they can locate in both the blood and skin, from a public health perspective it is most important that the microfilariae can be differentiated from *W. bancrofti* and *O. volvulus*.[2–5,25,30,38,119–122] The microfilariae of *M. ozzardi* can be easily distinguished from those of *W. bancrofti* by virtue of being smaller and having no sheath (Figure 3).[5,18,25,30,120] Living microfilariae of *M. ozzardi* can be obtained from skin snips and are not easily differentiated from *O. volvulus* by movement.[2–5,25,30,38,119–122] Their morphology is similar to the pathogenic *O. volvulus*, and it is especially important to distinguish these two species where they are sympatric, eg, in Amazonia onchocerciasis focus. Although morphological characters can be used to discriminate these two species,[38] difficulties have led to false reports of new onchocerciasis foci

and continue to pose a problem for onchocerciasis epidemiological monitoring in the Amazonia focus, which is now the last Latin America onchocerciasis focus where transmission is considered to be ongoing.[30,38,40,99,123,124]

Inmunodiagnosis

Immunological methods involve detection of either antibody or antigen. When a filarial parasite immunodiagnostic assay is detecting antibodies, enhanced specificity is often achieved by assessment of IgG4 rather than total IgG, as IgG4 antibodies are significantly elevated in microfilaria-positive individuals.[125] Although there is presently no effective antigen- or antibody-detecting immunological assay for diagnosis or epidemiological monitoring of mansonellosis infections, both kinds of tools have been developed for onchocerciasis and lymphatic filariasis disease-control programs, and have been tested for cross-reactivity with mansonellosis sera.[4,25,51,126–128] The reliability (specificity and sensitivity) of these immunodiagnostic tools for lymphatic filariasis and onchocerciasis has not been fully characterized for all of the tests, and the best-understood and most reliable tests are not necessarily the most convenient, and thus whether they should be employed to assist with mansonellosis research and diagnostics is questionable.[4,51,124,126–128] However, two recently published *M. perstans* studies used generic immunological filariasis assays to support light-microscopy-based diagnoses.[116]

Molecular diagnosis

Although DNA-based diagnostic tools can be used to detect and identify all of the known human filarial parasites, their most important utility to public health is their ability to differentiate skin infections of *M. streptocerca* and *M. ozzardi* from *O. volvulus* skin infections and *M. perstans* parasitemias from *L. loa* and *W. bancrofti* parasitemias.[51,126] DNA-based techniques for filarial parasite detection and identification have been proven to be both sensitive and specific, and have now begun to replace light-based microscopy in epidemiological surveys of mansonellosis.[4,49,51,52,114,129–133] Molecular diagnosis may be used to detect microfilariae in both peripheral blood and skin biopsies, and adult worms in other tissues.[4,51,52,114,129] PCR-based amplification of species-specific target sequences allows increasing diagnostic sensitivity compared with microscopic methods and reliable differentiation of samples taken from individuals living in coendemic areas.[51,52,114,127] In 2010, Tang et al[53] developed a popular nested PCR that could detect any form and life stage of filariae in the human host or vector. This assay uses universal filariae PCR primers to amplify a variable portion of filarial parasite ribosomal ITS1 DNA, and allows for the subsequent identification of species based on the size of the amplified fragment. This technique is used in conjunction with gel electrophoresis and/or Sanger sequencing, and allows for the characterization of previously unknown species (the *M. perstans* variant Deux was found in this way).[49,53,114,129] Recently, this rDNA ITS1-based method was turned into a single-step diagnostic by adapting it for real-time PCR.[113,131] This new assay allows for the identification of filarial parasite without the need for gel electrophoresis or Sanger sequencing, but does require more expensive reagents and infrastructure than standard PCR assays, and does not directly allow for the characterization of novel or unexpected filarial species.[113,131] Other methods (such as PCR–restriction-fragment-length polymorphism [RFLP]) that do not allow for the characterization of novel or unexpected filarial species, but require less infrastructure to support them and can differentiate a broad range of filarial species using universal primers with a combination of PCR and RFLP, have also been reported.[132,133] Despite requiring less complex infrastructural support than alternative methods, PCR-RFLP assays still require access to PCR reagents and a PCR machine, which can be a limiting factor for filarial parasite epidemiological studies.

Last year, the first DNA-detecting loop-mediated isothermal amplification (LAMP) filarial parasite assays were reported.[134,135] Although these assays have not yet been rigorously tested in the field or developed for the detection of *Mansonella* parasites, there is no theoretical reason they could not be adapted for mansonellosis research.[134,135] Requiring almost no infrastructural support, LAMP assays are a particularly appealing diagnostics option for research studies that need to be done in resource-limited settings.[51,134,135] For this reason, these new LAMP assays can be seen as a welcome development for filarial parasite research in general and an interesting new tool of research to develop for mansonellosis.[51,134,135]

Treatment

Among the three types of human mansonellosis, the one caused by *M. perstans* is usually regarded as the most difficult to treat.[4,18,23,25,116] Treatment studies have provided conflicting results, and there are at present no international guidelines on best treatment.[18,23,25,67,116,136–140] Few studies have recruited sufficient patients to provide statistically robust results to support their conclusions, and many are based on the treatment of one or two European or North American expatriates or tourists returning from endemic areas, who may not respond to treatments in the same way as residents of endemic areas.[4,23,25,116] The existence of genetically distinct strains of *M. perstans* (like Deux or strains that lack *Wolbachia*) complicates the formulation of treatment guidelines further, as it also possible that the efficacy of therapeutics varies between these strains.[60,66]

In one of the more robust *M. perstans* mansonellosis studies hitherto, conducted in the south of Chad, symptomatic subjects with evidence of *M. perstans* microfilariae in the peripheral blood were divided in groups and tested with the antiparasitic drugs diethylcarbamazine (DEC), mebendazole, ivermectin, praziquantel, thiabendazole, and DEC plus mebendazole.[137] DEC plus mebendazole proved to be the most effective antiparasitic treatment to reduce microfilaremia, and it is now one of the most used. Thiabendazole was second, with a single treatment significantly reducing microfilariae in blood. Ivermectin and praziquantel did not prove to be useful in the treatment of *M. perstans* infections. Mebendazole alone was seen to be a good alternative treatment and appeared to be more active than DEC alone in eliminating the infection.[137]

In another study carried out in Uganda, ivermectin alone, albendazole alone, and the two drugs in combination were assessed in three groups infected with *M. perstans*.[138] In this study, in concordance with the aforementioned study, microfilarial levels were unaffected by ivermectin or albendazole treatment when these drugs were used alone. Van den Enden et al[136] reached the same conclusion about albendazole treatment after finding that treatment had not decreased

microfilarial counts after a median follow-up period of 45 days. However, it has been found that when these drugs (ivermectin + albendazole) are used in combination, microfilarial loads decrease slightly after treatment.[136,139]

In contrast to conventional anthelmintic treatments, doxycycline has proved to be excellent, effective, and safe in the treatment of *M. perstans* infections.[4,67,116,139,140] However, the course of treatment over 6 weeks, which is necessary for this type of therapy, probably makes it impractical for control programs, although the fact that it appears to be curative makes it a very desirable therapeutic for travel medicine.[4,65,67] However, doxycycline treatment may not work on all *M. perstans* infections, as there is evidence of subspecies genetic diversity among *M. perstans* parasites and it is presently unclear whether all *M. perstans* strains harbor the *Wolbachia* endosymbiont.[60,66] This treatment's safety profile and all hitherto-published results on its efficacy, however, suggest that doxycycline treatment should be the first-choice treatment for perstans mansonellosis wherever it can be feasibly administered.[7,67,115,116]

The use of ivermectin for treating *M. ozzardi* has proven to be very effective against microfilariae in a number of studies.[3,23,25,29,139–143] A single dose of ivermectin given to infected adults was seen to reduce microfilariae densities and provide both short- and long-term reductions in *M. ozzardi* microfilaraemia.[29,142,143] Adverse events, however, have been seen in some patients with *M. ozzardi* treated with ivermectin.[143] Two elderly patients from Argentina developed serious adverse events that resembled a Mazzotti reaction following ivermectin administration, although the patients recovered without sequelae after 2–3 days.[143] DEC has little or no effect on microfilariae of *M. ozzardi*.[3]

Because of its effectiveness in the treatment of *M. ozzardi*, ivermectin has been used as a mansonellosis control tool in the Brazilian Amazon region.[139,140] The recent discovery of *M. perstans* in the northern regions of the Brazilian Amazon, however, suggests that this approach will not be effective everywhere.[50] As *M. ozzardi* are also known to harbor the endosymbiotic *Wolbachia*, it maybe that mansonellosis in this region can be tackled with a single anti-*Wolbachia* treatment regime using an antibacterial agent.[3,4,144] Although the six-week treatment course required for doxycycline-based therapies makes them impractical for filarial parasite-control programs, new anti-*Wolbachia* therapeutics with far shorter treatment courses (of 3–7 days) are in the pipeline and could make appealing treatment options throughout the whole Amazon region and beyond.[145–148] However, it is important that before such a measure is employed, efforts are made to determine whether this treatment interrupts transmission (like ivermectin treatment does). This is important to establish, as anti-*Wolbachia* treatments are not thought to kill *Mansonella* microfilariae directly and may thus not interrupt mansonellosis transmission as effectively as some of the alternative options.[67,115,117]

DEC and ivermectin have been used with good effect to treat *M. streptocerca* infection.[4,18,25,39,149] Fischer et al found that a single dose of 150 μg/kg body weight of ivermectin suppresses microfilaria for a year or more, with 46% of individuals displaying no detectable microfilaria on skin biopsy 1 year after treatment.[149] The most common treatment for *M. streptocerca* infections is DEC given at 6 mg/kg/day orally in three doses for 12 days.[139,140] At present, it is unclear whether *M. streptocerca* harbors a *Wolbachia* endosymbiont like *M. ozzardi* and *M. perstans*.[4,66] If it does, the possibility of using an anti-*Wolbachia* therapeutic like doxycycline for the treatment of *M. streptocerca* should also be investigated.[4,139,140]

Global burden

The high prevalence of asymptomatic cases and the lack of a universally agreed clinical profile for any of the three forms of mansonellosis infection has left many researchers in the field reluctant to even describe mansonellosis as a disease, with some authors conspicuously avoiding the word "disease" and some preferring to use the term "condition".[1–5,18,25] This prevailing perspective of mansonellosis as a benign parasitic infection with little or no direct effect on patients' well-being has left mansonellosis research trapped in a vicious circle of neglect. Mansonellosis research is unable to generate robust clinical data exactly because of the present paucity of robust clinical data makes funding mansonellosis research difficult for research councils to justify.

In addition to this, the symptoms currently directly attributed to mansonellosis are difficult to utilize in the modern metric systems used to assess disease burden and in the allocation of research funding. From the data presently available, it is impossible to assign any mortalities directly to mansonellosis infection and even difficult to assign the disability-adjusted life-years or years lost to disability, which have been used so effectively in the advocacy of attracting research funding to the 13 core neglected tropical diseases.[150–152] Recent surveys of the global burden of disease commissioned by a major tropical disease research fund have omitted mansonellosis from their analyses, which has surely resulted in the disease receiving a neglect that may be inappropriate.[151–154]

Even without a recognized disease burden, however, it is still clear that mansonellosis has an important role in

global health by indirectly interfering with the effectiveness of control programs targeting more serious diseases.[4,29,53] As mentioned earlier, the similarity between the microfilariae of *O. volvulus* with *M. streptocerca* (in Africa) and *M. ozzardi* (in Latin America) encountered in skin snips has led to false reports of onchocerciasis foci and misallocation of precious onchocerciasis-control resources.[39,40,120] Although we are unaware of published accounts of *M. perstans* in skin being confused with *O. volvulus*, the recent molecular detection of *M. perstans* DNA in skin snips on the island of Bioko (Equatorial Guinea) suggests that it could potentially have happened without being detected.[113] Similarly, mansonellosis parasites have been shown to interfere with some onchocerciasis immunodiagnostic assays and are suspected of interfering with the reliability of others.[51,120,124] In relation to lymphatic filariasis control, false-positive results with the Binax Now filariasis immunochromatographic test have recently been recorded in several independent studies carried out in central Africa. It is presently argued that the observed cross-reactivity is entirely attributable to *L. loa* infections, but a role for *M. perstans* in cross-reactivity has not yet been definitively ruled out.[128,151,152]

Beyond mansonellosis's direct and indirect (by raising morbidity in coinfection cases) disease burden and beyond even its role in interfering with the diagnostics used in other neglected tropical disease-control planning, it could also be negatively affecting global health by negatively impacting the efficacy of vaccine programmes.[154–156] It has been shown, for example, that certain lymphatic filariasis infections can affect the efficacy of some vaccines (including tuberculosis and HIV vaccines), and thus it is quite possible that mansonellosis infections could have a similar effect.[156] For this reason, some authors have discussed the possibility of auxiliary "deworming" programs to accompany vaccine programs as a way of potentially improving their efficacy.[154–156] Given the importance of vaccines as tools for infectious disease control and the extremely high prevalence of mansonellosis in infectious disease hot spots, the influence mansonellosis has on the efficacy of vaccine programs may in fact be the most important impact mansonellosis has on global health.[154–156]

Disclosure

The authors report no conflicts of interest in this work.

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