Mansonella ozzardi: a neglected New World filarial nematode

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Mansonella ozzardi (Nematoda: Onchocercidae) is an understudied filarial nematode, originally described by Patrick Manson in 1897, that can be transmitted by two families of dipteran vectors, biting midges (most of them members of the genus *Culicoides*) and black flies (genus *Simulium*). With a patchy geographic distribution from southern Mexico to northwestern Argentina, human infection with *M. ozzardi* is highly prevalent in some of the Caribbean islands, along riverine communities in the Amazon Basin, and on both sides of the border between Bolivia and Argentina. There is no clinical entity unequivocally associated with *M. ozzardi* infection, although fever, arthralgia, headache, cold lower extremities, and itchy cutaneous rashes are occasionally mentioned in case report series. More recently, ocular manifestations (especially keratitis) have been associated with mansonelliasis, opening an important area of investigation. Here, we briefly review the biology, epidemiology, pathogenesis, and clinical aspects of *M. ozzardi* infection and point to some existing knowledge gaps, aiming to stimulate a research agenda to help filling them.

Keywords: *Mansonella ozzardi*, Nematode, Microfilariae, Amazon, Pathogenesis, Diagnosis

Introduction

Mansonella ozzardi (Nematoda: Onchocercidae) is one of the several filarial nematodes that infect humans. This relatively unknown parasite has a patchy geographic distribution across Latin America and the Caribbean, from southern Mexico to northwestern Argentina. Most infected people, regardless of the parasite density, are asymptomatic or have few symptoms. As a consequence, infections with *M. ozzardi* usually remain undiagnosed and untreated. Ill-defined and unspecific symptoms such as fever, arthralgia, headache, cold lower extremities, and itchy cutaneous rashes are occasionally reported by patients, but whether they are caused by *M. ozzardi* infection remains to be determined. Nevertheless, ocular manifestations potentially associated with mansonelliasis, especially keratitis, have attracted substantial interest from ophthalmologists in recent years. Here, we summarize key biological, epidemiological, and clinical aspects of *M. ozzardi* infection. We explore recent developments in pathogenesis, laboratory diagnosis, and chemotherapy and discuss the potential public health impact of this highly prevalent but largely neglected New World parasite.

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Biological features

Three filarial nematodes of the genus *Mansonella* are known to cause human mansonelliasis: *Mansonella streptocerca*, which is endemic to Africa; *Mansonella perstans*, which is commonly found in Africa but also occurs in South America; and *M. ozzardi*, which is found exclusively in the Americas and the Caribbean islands.[1] Only humans appear to be naturally infected with *M. ozzardi*; African patas monkeys (*Erythrocebus patas*), but not chimpanzees, rhesus, capuchin, or squirrel monkeys, are susceptible to experimental infection with this nematode.[2]

Sir Patrick Manson (1844–1922) first described the microfilariae of *M. ozzardi* in the late 1890s, while examining the peripheral blood of Amerindians living in the interior of the former British Guyana.[3] The parasite, originally named *Filaria ozzardi* by Manson, was placed by Faust in the new genus *Mansonella* in the late 1920s.[4] Several decades later, Orihel and Eberhard described the elusive adult male and female worms recovered from experimentally infected patas monkeys.[2]

Natural infection with *M. ozzardi* begins with the bite of infected vectors, either biting midges (most of them of the genus *Culicoides*) or blackflies (genus *Simulium*), which deposits third-stage (L_3) larvae onto the skin of the human host (Fig. 1). These L_3 larvae undergo two further molts and develop into adult worms Adults are cylindrical in shape; the females measure 32–61 mm in length and 0.13–0.16 mm in diameter and the males

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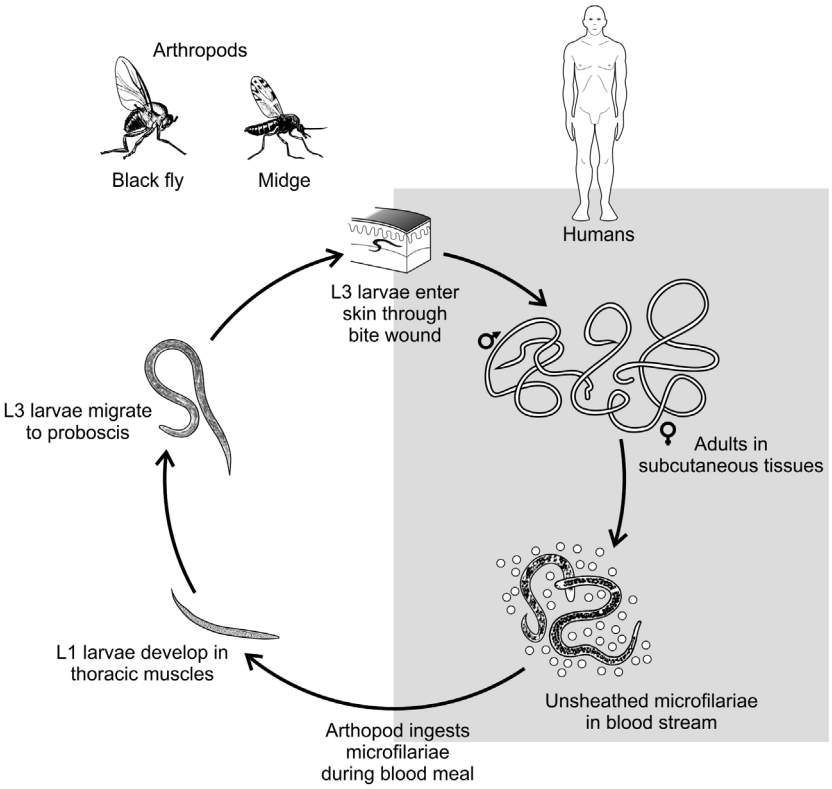

Figure 1 Schematic representation of the life cycle of *Mansonella ozzardi*. Modified from: Centers for Disease Control and Prevention. DPDx – Laboratory Identification of Parasitic Diseases of Public Health Concern. Mansonellosis. 2013. Available at: http://www.cdc.gov/dpdx/mansonellosis/index.html.

24–28 × 0.07–0.08 mm.[5] Their presumed habitat in humans remains uncertain; in experimentally infected patas monkeys, small numbers of adult *M. ozzardi* worms have been recovered from subcutaneous tissues, but not from the abdominal cavity or mesenteries.[2]

Unsheathed microfilariae with sharp tails are released by the viviparous female worms and reach the bloodstream. Circulating microfilariae are detected all day long, but moderate periodic fluctuation has been described in microfilarial density in the capillary blood of infected subjects.[6,7] This fluctuation is not necessarily synchronous, with different peak hours among hosts originating a pattern of crypto-periodicity that may be difficult to discern.[8] Microfilariae may also be occasionally found in the skin of infected subjects.[9–11] *M. ozzardi* microfilariae harbor maternally transmitted bacterial endosymbionts of the genus *Wolbachia*,[12] with potential immunologic and therapeutic implications that have been recently reviewed elsewhere.[13] The prepatent period in human infections is unknown, but in patas monkeys the first microfilariae are detected in the bloodstream 149–186 (mean, 168) days after subcutaneous inoculation of L_3 larvae.[2]

Microfilariae measure 207–232 (mean, 220) µm in length and 3–4 µm in maximum diameter, when formalin-fixed and stained with hematoxylin, and 185–214 (mean, 200) µm × 4–5 µm, when methanol-fixed and stained with hematoxylin or Giemsa.[5] The tip of the microfilaria's sharp tail is devoid of nuclei, while its anterior extremity has a cephalic space (7–9 µm) that ends at the point where the nuclear column begins. The 2–3 anterior-most nuclei are typically found in a single line just caudal to the cephalic space,[5] but occasionally two paired nuclei followed by a single nucleus are found in atypical *M. ozzardi* microfilariae described in Brazil[14] and Peru.[15]

The microfilariae of *M. ozzardi* are usually smaller than those of *Onchocerca volvulus* (that causes human onchocerciasis), which measure 186–286 (mean, 253) µm in length.[16] However, sizes may overlap, posing a major diagnostic challenge when unsheathed microfilariae are found in skin biopsies from communities in South America, where both species co-exist.[16,17] In Brazil, *M. ozzardi* occurs sympatrically with *O. volvulus* in some areas within the Amazonian onchocerciasis focus.[17–19] Further morphological[16] and molecular[19,20] analyses of skin-dwelling microfilariae are required to prevent misidentifying *M. ozzardi* as *O. volvulus* in these areas.

Similarly, *M. perstans* is also found in sympatry with *M. ozzardi* across the Amazon, in Southern Colombia,[21] Western Guyana,[22] and Venezuela.[23]

The microfilariae are ingested by the vectors (either biting midges of the genus *Culicoides* or black flies of the genus *Simulium*) during blood meals. Their subsequent development in the black flies, which have been described in detail by Tidwell and colleagues,[24] is briefly summarized here. The ingested microfilariae migrate within 2 h of the blood meal from the stomach wall, through the hemocoel, to the thoracic musculature. The larvae shorten, reaching

Table 1 Mansonella ozzardi vectors across Latin America

Region	Country	Biting midges	Black flies	Key references
North America	Mexico	*Culicoides furens*		Biagi et al.[31]
Central America	Panama		*Simulium sanguineum*	Petersen et al.[40]
Caribbean	Haiti	*C. furens*, *C. barbosai* *Leptoconops bequaerti*		Lowrie et al.[27–29]
	St. Vincent	*C. furens*, *C. paraensis?*		Buckley[25,26]
	Trinidad	*C. phlebotomus*		Nathan[30,32]
South America	Argentina	*C. paraensis*		Nathan and Wygodzinsky[42]
		C. lahillei, C. paraensis	*S. exiguum*	Shelley and Coscarón[43]
		C. debilipalpis, *C. lahillei*, *C. paraensis*		Veggiani-Aybar et al.[44]
	Brazil		*S. amazonicum*, *S. argentiscutum*	Cerqueira[34]
			S. oyapockense s.l. or *S. roraimense*	Shelley et al.[35–37]
	Colombia	*C. insinuatus*, *C. caprilesi*?	*S. amazonicum*, *S. argentiscutum* *S. sanguineum*	Tidwell et al.[24,33]
	Guyana		*S. oyapockense s.l.*	Nathan et al.[41]
	Suriname	*C. guttatus*?		Bruijning[96]
	Venezuela		*S. oyapockense s.l.*, *S. guyanensis*	Yarzábal et al. and González[38,39]

Modified from Shelley and Coscarón.[43]

the minimum length (123 μm) within 48 h. The first molt is usually completed 4.5 days after infection, leading to early second-stage larvae measuring 291 μm in length and 23 μm in width. Following the second molt, third-stage infective larvae are first observed between days 5 and 6, mostly in the head (in the proboscis), but also in the thorax and intestine of the vectors, and measure approximately 630 × 18 μm. These larvae can be transmitted to the definitive host during the blood meal.

One parasite, two families of vectors

The arthropod species that are currently known to transmit *M. ozzardi* are listed in Table 1, but data from several endemic settings remain incomplete or absent. Vectors belonging to two dipteral families are involved in transmission. Biting midges, members of the genus *Culicoides* and, less frequently, *Leptoconops* (Diptera: Ceratopogonidae), were first identified as vectors on several Caribbean islands and Mexico, while black flies of *Simulium* genus (Diptera: Simuliidae) were shown to transmit this parasite in Central and South America. The biting midge *Culicoides furens* was first shown to transmit this parasite on St. Vincent Island in the early 1930s.[25,26] Biting midges were later found to be *M. ozzardi* vectors on other Caribbean islands[27–30] and the Yucatan Peninsula of Mexico.[31] Subsequently, *Culicoides phlebotomus* was reported in endemic sites of Haiti[27] and Trinidad.[32] Several black flies, such as *Simulium sanguineum*, *Simulium amazonicum*, and *Simulium argentiscutum*, and the biting midge *Culicoides insiniatus* were reported in endemic sites of Colombia.[24,33] *S. amazonicum*, *S. argentiscutum,* and *Simulium oyapockense* have been implicated as the main vectors in the Amazon Basin of Brazil,[34–37]*Simulium sanchezi* in Venezuela,[38,39] *S. sanguineum* in Panama,[40], and *Simulium minusculum* in Guyana.[41] Romaña and Wigodzinsky described *Culicoides paraensis* as a vector of *M. ozzardi* in Tucumán province of northern Argentina.[42] Shelley and Coscarón reported that, in the Jujuy province of Argentina, *Culicoides lahillei* was the main vector, while *C. paraensis* and *Simulium exiguum* were secondary vectors.[43] *C. lahillei*, *Culicoides debilipalpis*, and *C. paraensis* were recently described as the main vectors of *M. ozzardi* in northwestern Argentina and southwest Bolivia.[44] Interestingly, *M. ozzardi* microfilariae were also found to develop in the musculature of other experimentally infected dipterans, such as *Anopheles aquasalis*, *Anopheles albitarsis*, and *Aedes aegypti*.[45]

The finding that vectors from different insect families are able to transmit *M. ozzardi* led to the hypothesis that highly divergent parasite populations with contrasting vector preference circulated in the Caribbean and the Amazon.[46] However, there is compelling evidence that *M. ozzardi* isolates from equatorial Colombia and subtropical Argentina can infect both black flies and biting midges.[33,43,47] Moreover, *M. ozzardi* strains from the Amazon and the Caribbean are morphologically identical at the ultrastructural level.[21]

A patchy geographic distribution

Human infections with *M. ozzardi* have been diagnosed exclusively in the Americas, from southern Mexico to northwestern Argentina (Fig. 2), with prevalence rates detected by conventional thick-smear microscopy ranging between zero and 46% in the general population (Table 2). Available data are fragmentary and should be interpreted with caution since studies differ according to the laboratory techniques used for diagnosis (which in turn differ in sensitivity), the age composition of surveyed populations, and several other factors that may affect prevalence estimates. Moreover, studies were carried out over several decades, and transmission levels may have varied with time in many endemic settings.

Figure 2 Presumed geographic distribution of *Mansonella ozzardi* across the Americas. Regions where this parasite has been reported (either in population-based surveys or case reports) and those that are contiguous with known endemic areas are shaded, but within each shaded region the transmission tends to be focal, with high-prevalence pockets surrounded with areas with no transmission. See main text and Table 2 for details.

Despite the parasite's versatility regarding vector preference, *M. ozzardi* transmission is clearly focal. Many transmission hotspots in the Amazon Basin map to indigenous communities, such as the Ticuna on Solimões river,[10] the Apurinã on Purus river,[48] and other Amerindian villages in Brazil[49] and Venezuela.[50,51] Endemicity levels vary widely among rural villages situated a few kilometers apart along the same rivers in the western Amazon Basin of Brazil.[52,53] Nevertheless, some riverside villages remain free of infection despite the presence of competent simuliid vectors.[54] The environmental factors that limit *M. ozzardi* spread beyond well-characterized hotspots along major rivers (mostly Solimões, Purus, Negro, and Orinoco) and their tributaries in the Amazon Basin remain largely undetermined, but a similarly patchy geographic distribution has been described for ceratopogonid-transmitted *M. perstans* in rural Africa.[55] In Haiti, where *C. furens* larvae breed in both brackish and freshwater while *C. barbosai* breeds exclusively in mangrove salty marshes, all major foci are located in coastal areas.[56] Transmission levels appear to have increased in recent years in known endemic areas of Brazil. For example, substantially higher positivity rates were detected by microscopy in riverine communities along the Purus river revisited in the 2000s[52,53,57] compared to the prevalence rates found at the time of the first survey, in the 1970s.[58] Relatively few cases of mansonelliasis have been documented in major cities of Amazonian Brazil,[59,60] but urban *M. ozzardi* transmission appears to occur in towns and small cities along the Solimões[61] and Acre[53] rivers.

The highest prevalence rates in northwestern Argentina are currently found in sites covered by subtropical mountainous rainforest (Yungas), such as Tartagal and San Ramón de la Nueva Orán, both in Salta province, and Libertador General San Martín and San Pedro de Jujuy, both in Jujuy province.[44] This is consistent with previous studies in the provinces of Jujuy [62,63] and Salta.[64] The number of *M. ozzardi* infections diagnosed by microscopy has declined sharply in northwestern Argentina since 1986, when malaria transmission started to decrease and active case detection of malaria parasite carriers became less intensive.[44]

Table 2 Prevalence of Mansonella ozzardi infection in selected populations, as determined by microscopic and molecular methods on blood samples

Country	Sites	No. samples examined	Age range (years)	Prevalence (%)	Diagnostic technique	References
Argentina	Tucumán Province (El Cercado, Orán, El Churqui, El Molino, La Aguadita and El Timbó) Salta Province (Tartagal and Peña Morada) Jujuy Province (Arroyo Colorado, Santa Clara, Santa Bárbara and San Pedro de Jujuy)	7141	31–80	31.3 (El Molino) 39.1 (Arroyo Colorado)	Thick-smear microscopy	Mühlens et al.[62]
Argentina	Salta Province (El Oculto)	29	Mean, 45	20.7	Thick-smear microscopy	Taranto and Castelli[97]
Argentina	Jujuy Province (Río Colorado, Quebarchal, Barroso, Candelaria, Normenta, Arrayanal, Marta, Sauces, Loma del Medio, San Borja and Trementinal)	107	≥5	41.1	Thick-smear microscopy	Remondegui et al.[63]
Argentina	Salta Province (Balderrama and Metán)	32	>40	50.0	Two methods combined[a]	Zaidenberg[64]
Argentina	Tucumán Province (Santa Ana)	92	Unspecified	57.6 50.0 27.2	Venous blood PCR Knott Membrane filtration	Degese et al.[98]
Argentina	Salta Province (Acambuco, Aguas Blancas, El Oculto, San Ramón de la Nueva Orán, Algarrobal, Pichanal, Embarcación, General Ballivian, General Mosconi, Tartagal, Aguaray, Campo Durán and Salvador Mazza) Jujuy Province (Palma Sola, Isla Chica, San Borja, San Pedro de Jujuy and Libertador General San Martín)	417	Adults	92.3 (Salta Province) 46.9 (Tartagal) 30.1 (San Ramón de la Nueva Orán) 85.9 (Jujuy Province) 56.4 (Libertador General San Martín) 20.0 (San Pedro de Jujuy)	Thick-smear microscopy	Veggiani-Aybar et al.[44]
Bolivia	Parapeti and Yuti rivers, Camiri, Cordillera Province (Guarani Indians and mestizos)	296	<1–81	26.0	Thick-smear microscopy	Bartoloni et al.[68]
Bolivia	Pilcomayo river, Villa Montes, Gran Chaco Province (Guarani Indians and mestizos)	298	<1–85	0.7	Thick-smear microscopy	Bartoloni et al.[68]
Brazil	Solimões river, Tabatinga (Ticuna Indians)	800	All ages	45.7	Thick-smear microscopy	Moraes et al.[10]
Brazil	Içana river (Baniwa Indians)	24	>10	62.5	Thick-smear microscopy	Lawrence et al.[49]
Brazil	Marari river (Yanomama Indians)	11	>10	0	Thick-smear microscopy	Lawrence et al.[49]
Brazil	Solimões river (Ticuna Indians)	56	>10	30.3	Thick-smear microscopy	Lawrence et al.[49]
Brazil	Juruá river tributaries (Kanamari Indians)	82	>10	1.2	Thick-smear microscopy	Lawrence et al.[49]
Brazil	Purus river (Jaminawa Indians)	15	>10	0	Thick-smear microscopy	Lawrence et al.[49]
Brazil	Javari river (Marubo Indians)	20	>10	5.0	Thick-smear microscopy	Lawrence et al.[49]
Brazil	Juruá river tributaries (Kashinawa Indians)	37	>10	0	Thick-smear microscopy	Lawrence et al.[49]
Brazil	Envira river (Katukina Indians)	42	>10	0	Thick-smear microscopy	Lawrence et al.[49]
Brazil	Tacutu, Maú, Surumu and Cotingo rivers, western Roraima State (Makuxi and Wapixana Indians, 15 indigenous communities)	652	All ages	3.2	Thick-smear microscopy	Moraes et al.[37]
Brazil	Purus river, Pauini (6 indigenous communities)	169	2–71	28.4	Thick-smear microscopy	Medeiros et al.[48]
Brazil	Ituxi river, Lábrea (12 riverine communities)	129	≥2	30.2	Thick-smear microscopy	Medeiros et al.[66]
Brazil	Purus river, Boca do Acre (9 riverine communities)	282	≥2	27.3	Thick-smear microscopy	Medeiros et al.[52]
Brazil	Pauini river, Pauini (5 riverine communities)	177	2	24.9	Thick-smear microscopy	Medeiros et al.[18]
Brazil	Purus rivers, Pauini (30 riverine communities)	744	≥2	24.6	Thick-smear microscopy	Medeiros et al.[18]
Brazil	Purus river, Lábrea (23 riverine communities)	694	≥2	20.7	Thick-smear microscopy	Medeiros et al.[57]

(*Continued*)

Table 2 (*Continued*)

Country	Sites	No. samples examined	Age range (years)	Prevalence (%)	Diagnostic technique	References
Brazil	Solimões river, urban area of Coari	1069	All ages	10.2	Thick-smear microscopy	Martins et al.[61]
Brazil	Solimões river, rural area of Coari (10 riverine communities)	664	All ages	18.4	Thick-smear microscopy	Martins et al.[61]
Brazil	Mamoré, Madeira, Guaporé, Machado and Preto rivers (urban populations and riverine communities)	4452	>5	0	Thick-smear microscopy	Basano et al.[54]
Brazil	Tefé river, Tefé (11 riverine communities)	300	2–82	6.3	Thick-smear microscopy	Medeiros et al.[99]
Brazil	Acre river, Porto Acre and Vila Antimary	217	>18	12.9	Knott	Adami et al.[53]
Brazil	Antimary river (two riverine communities)	78	>18	1.3	Knott	Adami et al.[53]
Brazil	Purus river, Boca do Acre (2 riverine communities)	60	>18	60.0	Knott	Adami et al.[53]
Brazil	Solimões river, Codajás (riverine communities)	109	All ages	44.9 25.7	Venous blood PCR Thick-smear microscopy	Medeiros et al.[87]
Brazil	Solimões river, Coari (riverine communities)	105	All ages	44.8 29.5 22.9	Venous blood PCR Capillary blood (FTA) PCR Thick-smear microscopy	Medeiros et al.[87]
Brazil	Solimões river, Codajás (7 riverine communities)	245	All ages	10.2	Thick-smear microscopy	Medeiros et al.[100]
Brazil	Solimões river, Coari 5 (riverine communities)	127	All ages	22.0	Thick-smear microscopy	Medeiros et al.[100]
Colombia	Amazonas river, Comisaría del Amaznonas, Letícia (several indigenous peoples and mestizos)	535	>10	47.1	Knott	Kozek et al.[101]
Colombia	Amazonas river, Comisaría del Guainía, Puerto Inírida (several indigenous peoples and mestizos)	604	All ages	20.0	Knott	Kozek et al.[102]
Guyana	Six districts country wide (indigenous communities)	9506	All ages	1.5	Thick-smear microscopy	Orihel[22]
Haiti	Bayeux, north coast	1165	All ages	16.1	Thick-smear microscopy	Raccurt et al.[103]
Haiti	Corail, Grande Anse region, north coast	462	All ages	16.5	Thick-smear microscopy	Raccurt et al.[56]
Mexico	Northern Yucatan Peninsula	296	All ages	61.1	Thick-smear microscopy	Biagi et al.[31]
Panama	Chucunaque river, Darien (villages of Morti, Uala and Membrillo)	312	All ages	67.5 (Morti) 18.3 (Uala) 10.9 (Membrillo)	Thick-smear microscopy	Petersen et al.[40]
Peru	Amazonas river, Iquitos (periurban villages)	433	All ages	1.4	Thick-smear microscopy	Arróspide et al.[74]
Peru	Santa Maria de Nanay, Alto Nanay district, Loreto	134	All ages	47.8	Thick-smear microscopy	Vargas et al.[67]
Trinidad	Blanchisseuse, Northern Range montains (coastal community)	602 (1980) 348 (1992) 348 (1992) 384 (1992)	All ages	23.3 (1980) 21.5 (1992) 19.2 (1992) 11.8 (1992)	Two methods combined[b] Two methods combined[b] Thick-smear microscopy Membrane filtration	Chadee et al.[84]
Venezuela	Southwestern Bolívar State (8 indigenous communities)	139	All ages	57.6	Thick-smear microscopy	Godoy et al.[104]
Venezuela	Orinoco river, Southeastern Orinoquia (13 indigenous communities)	806	>10	22.2	Thick-smear microscopy	Medrano et al.[51]
Venezuela	Orinoco and Negro basins, Amazonas and Bolívar State (17 riverine communities)	1057	Unspecified	9.9	Knott	Gómez and Guerrero[105]

[a]Thick-smear microscopy and Knott combined.
[b]Thick-smear microscopy and membrane filtration combined.

The prevalence of *M. ozzardi* microfilaraemia in most endemic communities in the Amazon and the Caribbean typically increases with age, being highest in middle-age adults.[10,44,48,49,53,56,60,65–67] There is also evidence that average microfilaria counts, among microscopy-positive subjects, increase with age,[48,68] consistent with increased exposure in adults leading to frequent superinfection in the absence of effective acquired immunity. Adult males are more often affected than females,[18,44,48,52] with greater average microfilaremias in males,[48] suggesting that the risk of infection may be associated with occupation-related exposure (e.g. subsistence farming and fishing). Gender-related differences in the prevalence of infection, which are more pronounced in the adult population, are also seen in perstans mansonelliasis, lymphatic filariasis, and onchocerciasis in Africa.[55] In the Chaco region of Bolivia, however, similar prevalences of infection with *M. ozzardi* are found in males and females.[68] The close proximity between individuals' dwellings and main rivers is another well-recognized risk factor for infection.[58,61]

Is M. ozzardi entirely harmless?

Unspecific symptoms and clinical signs classically associated with ozzardi mansonelliasis include fever, articular pain, headache, cold lower extremities, cutaneous rashes and lymphadenopathy.[53,69,70] However, available descriptions of clinical symptoms are mostly derived from relatively small case series with no control group in which other infectious and noninfectious conditions have been explicitly ruled out. Eosinophilia is commonly observed, being reversed after treatment.[70,71] Deposition of circulating immune complexes, which have been detected in *M. ozzardi* infection,[72] might trigger articular inflammation and pain. However, not all community-based surveys revealed significant associations between clinical manifestations and the presence of infection.[68,73,74] As noted by Bartoloni and colleagues,[68] age may be a strong confounder of this association. In unadjusted analysis, they found a significant association between infection and unspecific clinical manifestations such as arthralgia, headache, and pruritus. However, they argue that this association may have been confounded by age since the prevalence of infection and that of unspecific symptoms in the general population increase with age. No association between clinical manifestations and *M. ozzardi* infection in the Chaco region of Bolivia was observed after Bartoloni and colleagues adjusted their analysis for age and gender.[68]

Since the early 2000s, ocular lesions have been described in *M. ozzardi*-infected subjects in indigenous and non-indigenous communities along the Negro and Solimões rivers in the Amazon Basin of Brazil.[75–77] No *O. volvulus* transmission has been documented in these areas[17] and in two of these studies all subjects tested negative for *O. volvulus* microfilariae in skin biopsies.[75,76] Garrido and Campos first described multiple nummular infiltrates in the cornea, with ≤2 mm in diameter, in 55% of 140 infected subjects (mostly indigenous) from the upper Negro river, with 2–8 lesions per eye.[75] None of the 358 uninfected subjects examined had similar corneal lesions. Most lesions were peripheral and did not affect the visual acuity of affected subjects.[75] A subsequent cross-sectional survey was carried out in riverine communities in Coari, along the Solimões river, where the overall prevalence rate of ozzardi mansonelliasis is estimated at 19%.[76] Corneal lesions were diagnosed in 15% of 95 infected subjects; punctate keratitis accounted for most (12 of 14) corneal lesions diagnosed, with sclerosing and nummular keratitis being found in only one subject each.[76] No microfilariae were found by microscopy or polymerase chain reaction (PCR) in conjuntival and limbal biopsies of five keratitis patients and corneal biopsies of three patients.[76] Finally, another cross-sectional survey in Coari found corneal lesions in 14 of 56 (25%) microfilaremic subjects and 8 of 156 (5%) uninfected controls.[77] These authors also provided the first report of *M. ozzardi* microfilariae identified in the cornea. They found, by confocal microscopy, linear lesions consistent with *M. ozzardi* microfilariae in the cornea of seven subjects; two of them had microfilariae detected on Giemsa-stained smears prepared with blood from the limbal conjuntiva.[77]

Although there is no definitive evidence that migrating *M. ozzardi* microfilariae can directly cause corneal lesions, a careful eye examination is recommended to be part of the routine clinical care of infected subjects, regardless of any symptoms. In areas of Central and South America where *M. ozzardi* and *O. volvulus* co-occur, skin biopsies are mandatory to rule out onchocerciasis as a cause of corneal lesions. Moreover, further studies are required to determine the extent to which the corneal lesions so far described are associated with visual symptoms and reduced visual acuity and whether lesions are responsive to therapeutic interventions to suppress microfilaremia and reduce ocular inflammation.

Chronic infections with tissue-invasive helminths typically affect immune responses to co-infecting pathogens by creating an immunoregulatory environment dominated by interleukin (IL)-10 and transforming growth factor (TGF)-β. The impact of filarial infections on antimalarial immunity has been characterized in detail in Africa, with an IL-10-dependent decrease in IL-12p70, interferon (IFN)-γ, and IFN-γ-induced protein 10 (IP-10) responses upon T-cell stimulation with malarial antigens,[78–80] but no data are available for chronic, mostly undiagnosed and untreated *M. ozzardi* infections in the Amazon.

Microscopic and molecular diagnosis

Most infections with *M. ozzardi* have been diagnosed by microscopic examination of Giemsa-stained thick blood smears. Because malaria and ozzardi mansonelliasis co-occur in several endemic settings, microfilariae are often found on thick smears originally prepared for malaria diagnosis[67], and sometimes co-infection with *Plasmodium* parasites were reported.[62,67,81]

As microfilariae circulate in the peripheral blood all day long, diagnostic capillary or venous blood samples can be obtained at any time. Concentration methods are required to diagnose low-density microfilaremias. A widely used concentration technique was originally described by Knott in 1939.[82,83] It involves mixing 5 ml of anticoagulated venous blood with 50 ml of a 2% formalin solution in a polystyrene tube and recovering microfilariae in the sediment after a brief centrifugation at 400*g*. A carefully collected aliquot of the sediment is used to prepare a thick smear, which is fixed with methanol, stained with Giemsa and examined under a microscope.

Alternatively, polycarbonate membrane filtration may be used, allowing for the examination of relatively large blood volumes. Anticoagulated venous blood (up to 10 ml) is diluted in 0.85% saline solution or phosphate-buffered saline and filtered through a 13 or 25 mm polycarbonate membrane (pore size, 3 µm) adapted to a sterile syringe. After this filtering procedure, followed by several washes with saline solution, the wet membrane is placed on a glass slide, fixed with methanol, stained with Giemsa or hematoxylin, and examined for retained microfilariae under 10–40× magnification. A survey in Trinidad showed an 1.6 × higher diagnostic sensitivity of thick-smear microscopy (using 20-µl finger-prick blood samples) compared to membrane filtration (5 ml of venous blood filtered through 25-mm Nuclepore filters with 3-µm porosity).[84]

Although *M. ozzardi* microfilariae can be found in the skin of infected subjects, skin biopsies should be obtained and examined for diagnostic purposes only in areas where ozzardi mansonelliasis and onchocerciasis are known or suspected to co-occur.[9–11] *M. ozzardi* microfilariae have also been found in the ascitic fluid of a patient.[85]

Molecular diagnosis may be used to detect *M. ozzardi* microfilariae in the peripheral blood, skin biopsies and other tissues. PCR-based amplification of species-specific target sequences allows for increased diagnostic sensitivity, compared with microscopic methods, and reliable differentiation between *M. ozzardi* and co-endemic filarial species such as *O. volvulus* and *M. perstans*.[19,20,86,87]

Key factors that determine the diagnostic sensitivity of PCR include sample storage conditions prior to DNA extraction, sample volume, and methods used to isolate DNA.[87] The PCR method described by Tang and colleagues[19] was 1.5 × more sensitive when DNA templates were prepared from 2.5-µl of venous blood, compared with those isolated from 0.6 µl of dried blood from FTA membranes.[87] Moreover, venous blood-based PCR was 1.8 × more sensitive than conventional thick smear microscopy (one smear examined per subject).[87]

Therapeutic approaches

Unlike most other filarial species, diethylcarbamazine (DEC) has little or no effect on microfilariae of *M. ozzardi*.[88] Accordingly, DEC-based mass chemotherapy (montly doses of 6 mg/kg of DEC citrate over 12 months administered between 1980 and 1981) has eliminated *W. bancrofti* from a rural community of Trinidad, but had virtually no impact on the local prevalence of *M. ozzardi* infection.[84] In contrast, the daily administration of levamisole (150 mg/day, adult dose) for 2–3 months has been found to suppress *M. ozzardi* microfilaremia in a limited number of patients.[70]

Ivermectin (one single dose of 0.14–0.2 mg/kg) is currently the treatment of choice,[89,90] although it remains uncertain whether it has any effect against adult worms. A single dose of 0.15 mg/kg of ivermectin has recently been reported to suppress *M. ozzardi* microfilaremia for at least 12 months in 53 subjects from Brazil, suggesting some effect on female worm survival or fertility.[71] Adverse effects that are reminiscent of Mazzotti reaction,[91] such as fever, chills, malaise, headache, arthralgia, dizziness, and dyspnea, have been often observed after the administration of ivermectin, but nearly all patients recover rapidly without specific therapy.[71,89,92] The severity of post-treatment reaction appears to correlate positively with pretreatment microfilarial density in *O. volvulus*[91] and *W. bancrofti*[93] infections, but whether this holds for *M. ozzardi* remains to be determined.

Because *M. ozzardi* harbors the endosymbiotic bacteria *Wolbachia*,[12] doxycycline may be an effective therapy to eliminate adult worms,[94] as recently shown for *M. perstans*.[95] However, no trials with doxycycline (either alone or in combination with ivermectin) have been conducted for *M. ozzardi* infection. Similarly, there are no data regarding the therapeutic efficacy of mebendazole or albendazole against *M. ozzardi* microfilariae or adult worms, but these benzimidazoles appear to be of poorly effective against *M. perstans*.[55]

Conclusions

Although infections with *M. ozzardi* are highly prevalent in areas of the Caribbean, the Amazon, and on both sides of the border between Bolivia and Argentina, several knowledge gaps persist. For example, there is no clinical entity unequivocally associated with human infection with *M. ozzardi*, but corneal lesions putatively caused by migrating microfilariae are a major reason for concern. It remains to be examined whether chronic mansonelliasis may exert a strong immunomodulatory effect, as many other helminthic infections. The habitat of adult worms in human hosts is uncertain and more affordable experimental models are surely needed to scrutinize the parasite's biology. Potential vector species have been carefully characterized in some Caribbean islands, parts of the Amazon and northern Argentina, but little is known for most other endemic settings. Despite recent evidence for ivermectin efficacy against microfilariae, it remains unclear whether this antihelmintic, either alone or combined with other drugs, is able to kill adult worms. These unknowns render *M. ozzardi* a typical neglected parasite that affects mostly poor rural populations across Latin America and the Caribbean.

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