

***Mansonella ozzardi* in the municipality of Tefé, Amazonas, Brazil, 60 years after the first report: an epidemiologic study**

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*The prevalence of mansonelliasis was studied in the municipality of Tefé, state of Amazonas, Brazil. The prevalence (thick blood smear method) was 13.6% (147/1,078), higher in the Solimões River region (16.3%) than in the Tefé River region (6.3%). In the sampled communities in the Solimões River region, a higher density of cases was observed, as indicated by a kernel analysis (odds ratio 0.34; 95% confidence interval: 0.20-0.57). Males had a higher prevalence ($\chi^2 = 31.292$, $p < 0.001$) than women. *Mansonella ozzardi* prevalence was higher in retirees and farmers (28.9% and 27%, respectively). Prevalence also significantly increased with age ($\chi^2 = -128.17$, $p < 0.001$), with the highest numbers occurring in persons older than 67 years.*

Key words: filariasis - mansonelliasis - *Mansonella ozzardi* - Amazonia

Mansonella ozzardi is one of the etiological agents of mansonelliasis, an endemic filaria in the Americas and Caribbean Islands (Shelley & Coscarón 2001). It is a poorly understood filarial species, without a well-defined pathogenicity, presenting asymptotically or with few symptoms in infected individuals or with obvious clinical manifestations (fever, joint pain, adenitis, headache, coolness of the lower extremities) (Batista et al. 1960, Tavares 1981). In addition, corneal lesions have also been reported in possible association with the parasitic infection (Vianna et al. 2013). Certain symptoms are shared with other diseases, such as malaria, confusing the diagnosis in endemic areas. To date, only the Simuliidae have been identified as vectors in Brazil (Medeiros et al. 2009, Martins et al. 2010).

M. ozzardi has a broad distribution in the state of Amazonas (AM), Brazil. It is found along the Solimões and Purus Rivers and the Negro River and its tributaries (Rachou 1957, Medeiros et al. 2009, Martins et al. 2010). Since the first records of its occurrence, it has been observed that the prevalence remains high in certain areas (Solimões River, municipality of Coari: 10% and 18.4%) (Rachou 1957, Martins et al. 2010), whereas sudden increases have occurred in other areas (Purus River, municipality of Boca do Acre: 0.2% and 27.3%; municipality of Lábrea: 0.4%, 4.4% and 20.7%) (Rachou 1957, Shelley 1975, Medeiros et al. 2009, 2011).

This study was conducted in the middle Solimões River region in the municipality of Tefé, AM. This is an

endemic region for *M. ozzardi*. The parasite was found during an epidemiological investigation in the 1950s that demonstrated prevalence between 2.5% and 18.7% (Rachou 1957). Due to the presence of oil and natural gas extracting companies, there is a continuous flow of population into this municipality from several Brazilian areas. Such human movement may allow the filaria to spread to nonendemic areas. Given this information, a broad inquiry was made in the region of the Solimões and Tefé Rivers to provide an updated epidemiological profile of *M. ozzardi* in Tefé.

This study was performed in 29 rural communities selected with the convenience sampling method in the Solimões and Tefé River regions in Tefé (3°21'S 64°43'W) (Fig. 1A). The municipality has 61,453 inhabitants (urban population = 50,069, 81.5%; rural population = 11,384, 18.5%) (IBGE 2010). The region is covered by native primary forest and has extensive flooded areas during the rainy season. Rural populations are located along the rivers in small villages. The economic base of these small communities consists of fishing, agriculture and livestock farming. The required sample size was calculated on the basis of an estimated *M. ozzardi* prevalence of 18.4% (Martins et al. 2010), an error of 20% and a confidence interval (CI) of 95%. Individuals participated in this study after giving informed consent. Within each community, individuals three years of age or older who underwent blood sampling were included. In total, 1,083 individuals (9.5% of the rural population) were examined by the Rapid Epidemiological Mapping test for microfilariae of *M. ozzardi* in April, August, September, October and November of 2012. We collected demographic (age, gender and profession) and geographic data (name of the village and geographical location).

M. ozzardi prevalence was measured by thick blood smears obtained by digital puncture. Two drops of blood (approximately 60 μ L) were collected from each individual and placed on a slide. After air-drying, the slides were

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stained with Giemsa and observed under light microscopy (200X/400X magnification). If the blood smears were positive, the mf was identified following Post et al. (2003). Additionally, *M. ozzardi* mf were also submitted to a new identification protocol using the polymerase chain reaction (SLB Luz, unpublished observations). The study protocol was approved by the Research Ethical Committee of the Tropical Medicine Foundation Dr Heitor Vieira Dourado (protocol 1504/10), in Manaus, AM. Informed consent was obtained from all adults and from the parents of the minors who participated in the study.

The data were analysed using Generalised Linear Models with R software (R Development Core Team 2010). To verify the differences in the prevalence of *M. ozzardi* between genders, activities and age groups, we performed an ANOVA followed by an analysis of contrasts as appropriate. Furthermore, odds ratios (OR) and 95% CI were determined for each group using Epicalc. Kernel mapping was used to analyse the spatial distribution of the intensity and prevalence of cases and to identify high-risk areas (hot spots) for *M. ozzardi*.

In total, 1,083 individuals were examined (average age 28.3 ± 19.5 , range 3-93 years) in 29 communities on the Solimões and Tefê Rivers, representing 9.5% of the rural population of Tefê. The male:female ratio was 0.80 (484/1,083: 44.7% male; 599/1,083: 55.3% female).

The overall prevalence of *M. ozzardi* was 13.5% (147/1,083). The prevalence in the Solimões River communities was 16.3% (128/783), whereas it was 6.3% (19/300) in the Tefê River communities, a significant difference (ANOVA: $\chi^2 = 23.259$, $p < 0.001$). A kernel analysis indicated that the areas with a higher intensity and prevalence of cases were located near the Solimões River (Fig. 1B, C); the Solimões River also had a higher OR relative to the Tefê River (OR: 0.34; 95% CI: 0.20-0.57) (Fig. 2A). In the area of the Solimões River, in contrast to the Tefê River, the communities are larger

and closer to each other, with a higher population density. This proximity favours a higher transmission rate of filariae by Simuliidae. The higher prevalence in the Solimões River area is facilitated by the presence in the area of the simuliid black fly *Cerqueirellum argentiscutum*, which transmits the parasite in the area (Martins et al. 2010). Those observations are strengthened by the finding that the risk ratio was higher in the Solimões River area and by the kernel analysis, which showed hot spots in the Solimões River communities. The prevalence (16.3%) in the Solimões River communities in Tefê is close to that found along the same river in Coari, between 17.1-17.8% (Rachou 1957) or 18.4% (Martins et al. 2010) and in Codajás, where the prevalence was 23.9% (Rachou 1957). In certain communities, the prevalence is still high after several decades. In Missão, Caiambé and Jutica, the prevalence found by this study was 11.3%, 18.2% and 30%, respectively. Previously, the prevalence was 13.3%, 17.5% and 18.7%, respectively (Rachou 1957). Similar findings were obtained in other municipalities of AM: in Coari, during the 1950s, it was 10% and it was 17.1% and 17.8% in the river communities (Rachou 1957). More recently, prevalence of 10.2% was found for urban zona of Coari and 18.4% and 26.4% for the river communities (Martins et al. 2010, Vianna et al. 2013). In the Purus River municipalities, such as Lábrea, prevalence of 0.4%, 4.4% and 5.9% were found (Rachou 1957, Shelley 1975, Tavares 1981) and, more recently, 30.2% and 20.7% (Medeiros et al. 2008, 2011). The stability and increase in prevalence found both in Tefê and others municipalities over the years is primarily a result of a lack of treatment policies, resulting in persistent infection rates and continuous transmission. It is possible that this permanent transmission cycle maintains the stability of infection because many individuals may become infected repeatedly over time, resulting in permanent microfilaria production and compensating for

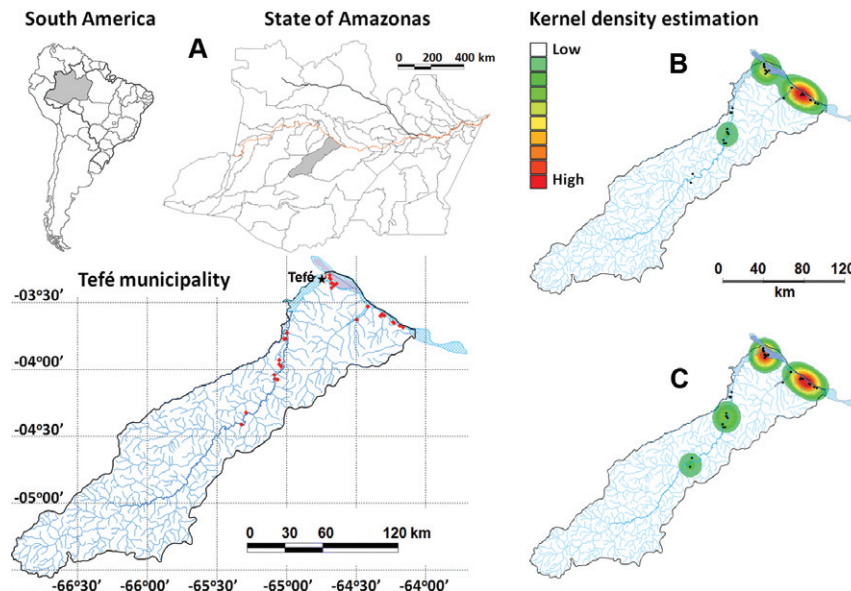


Fig. 1A: map of study area location. The red points indicate study localities. The black star indicates the municipality of Tefê, state of Amazonas, Brazil; B: Kernel density estimation for cases; C: Kernel density estimation for prevalence.

TABLE
Mansonella ozzardi prevalence by gender, age group, occupation and risk factors for filaria in the municipality of Tefé, state of Amazonas, Brazil

Variable		Positive/total [n/n (%)]	ANOVA	
			p	Analyse of contrast
Total		147/1,083 (13.5)		-
Gender	Female	51/599 (8.5)	p < 0.001	-
	Male	96/484 (19.2)		-
Age group (years)	2-9	3/178 (1.6)	p < 0.001	a
	10-18	15/271 (5.5)		b
	19-27	15/164 (9.1)		b
	28-37	28/159 (17.6)		c
	38-47	23/107 (21.5)		c
	48-57	26/91 (28.6)		c
	58-67	18/63 (28.6)		c
	> 67	19/50 (38)		d
Activity	Farmer/fisherman	69/255 (27)	p < 0.001	a
	Housewife	30/232 (12.9)		b
	Student	18/437 (4.1)		c
	Retired	24/83 (28.9)		d
	Other	6/76 (7.9)		b

different letters indicates statistical difference (p < 0.05).

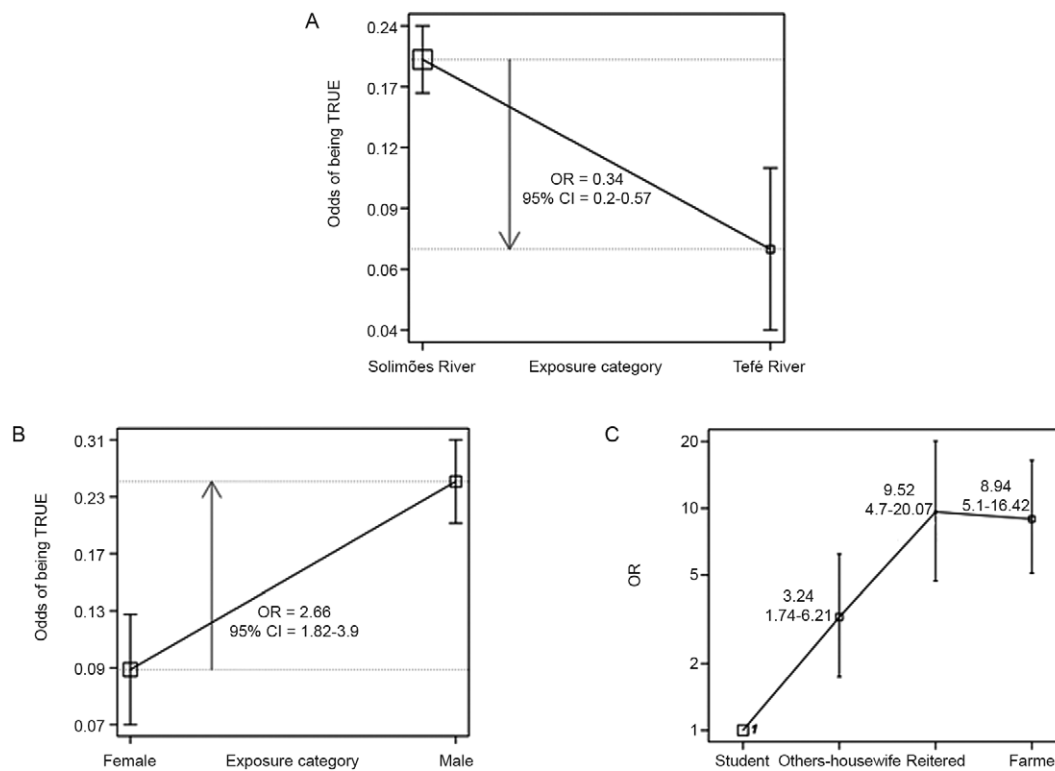


Fig. 2: odds ratio (OR) between Solimões and Tefé Rivers (A), between gender (B) and among different occupations (C) in the municipality of Tefé, state of Amazonas, Brazil. CI: confidence interval.

the decreased fertility and mortality of older worms. The lifespan and reproductive cycle of adult *M. ozzardi* are unknown. However, Orihel et al. (1993) observed that in monkeys, infection can persist for up to 212 weeks and that microfilaremia constantly increased at the beginning of infection, reaching its highest level at 20 weeks and then slowly decreasing to stable levels for up to 48 weeks. Subsequently, microfilariae disappeared from the blood and occasionally reappeared in low numbers.

M. ozzardi was significantly higher in males than in females (Table) (ANOVA: $\chi^2 = 31.292$, $p < 0.001$). Furthermore, the prevalence of infection also differed significantly among age groups. An analysis of contrasts identified four groups: group 1 (0-9 years), group 2 (10-27 years), group 3 (28-67 years) and group 4 (> 67 years) (ANOVA: $\chi^2 = -117.11$, $p < 0.001$). A significant difference was found in *M. ozzardi* prevalence between different occupations (ANOVA: $\chi^2 = -105.37$, $p < 0.001$), with the highest prevalence in retirees and farmers (28.9% and 27%, respectively). The OR analyses for each variable (i.e., gender, occupation and age group) indicated the following: (i) men had a higher risk ratio than women (OR: 2.66; 95% CI: 1.82-3.90) (Fig. 2B), (ii) according to occupation, the highest risk of acquiring the infection was in retirees (OR: 9.52, 95% CI: 4.70-20.07) (Fig. 2C) and individuals older than 67 years had a high OR relative to all other age groups (OR: 33.89, CI 95%: 9.21-189.33).

The difference between genders is due to the higher exposure of males to simuliid bites, a consequence of occupational exposure, as observed by other authors (Medeiros et al. 2009, 2011, Martins et al. 2010, Adami et al. 2014). The degree of exposure to vectors is also the factor that directly influences prevalence levels by age group, indicating that infection levels normally increase proportionally with age (Bartoloni et al. 1999, Medeiros et al. 2011). This trend was confirmed in our study by the comparative analysis and OR, which demonstrated that individuals older than 67 years had a risk ratio up to eight times higher than that in other age groups. Finally, in terms of working activity, the high prevalence found in retired people may be related to occupation because most are farmers and normally continue working even after retirement, with continued exposure and multiple opportunities for reinfection. Multiple exposures to reinfection are also reflected by age; the older the person is, the higher the risk of carrying the infection. Bartoloni et al. (1999) suggests that the high level of microfilaremia in adults is due to the continuous succession of superimposed infections. This hypothesis is strengthened by observations by other authors (Martins et al. 2010, Medeiros et al. 2011) that also demonstrated a higher prevalence in farmers and retirees.

This paper confirms that the prevalence in Tefé is still high even after 60 years based on the same diagnostic method used by Rachou (1957). If more sensitive methods, such as the Knott method and molecular diagnostics, were used, the observed prevalence should be much higher. In Codajás, for example, the prevalence based on blood films was 23.5%, but the observed prevalence increased to 41.4% when the Knott method was used (Moraes 1958).

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