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Mansonella perstans and *Plasmodium falciparum* Co-infection in the Akonolinga Health District, Centre Region, Cameroon

Leopold Gustave Lehman[1], Martin Gael Oyono[2], Samuel Fosso[3], Charles Félix Bilong Bilong[2]

[1]Faculty of Sciences, University of Douala, Douala, Cameroon

[2]Faculty of Sciences, University of Yaoundé I, Yaoundé, Cameroon

[3]Faculty of Medicine and Biomedical Sciences, University of Yaoundé I, Yaoundé, Cameroon

Email address:
oyono.martingael@gmail.com (M. G. Oyono)

Abstract: *Mansonella perstans* and *Plasmodium falciparum* are among the most common human parasites in Sub-Saharan Africa. They pass through the bloodstream during their life in human host. This study aimed at determining the prevalence, determinants of co-infection with *M. perstans* and *P. falciparum* and their possible interaction mechanism. A cross-sectional study was conducted in the Akonolinga Health District among pupils. Each of them was screened for the presence of peripheral blood parasites stages using Giemsa-stained thick and thin blood films. Socio-demographic information was documented using a questionnaire forms. A total of 416 pupils aged 4-15 years (average: 9.17 ± 0.27) were recruited. The overall prevalence was 4.32% and 37.26% for *M. perstans* and *P. falciparum* respectively, and prevalence of co-infection was 1.92%. Mean parasite density was 508.7 ± 310.3 (min: 430 - max: 1300) µF/ml for *M. perstans* and 5240.38 ± 2037.42 (min: 857 – max: 10400) T/µl for *P. falciparum*. Risks of single infections (aOR = 0.46, $P = 0.0264$ for *M. perstans* and aOR = 0.64, $P = 0.0432$ for *P. falciparum*) and co-infection (aOR = 0.10, $P = 0.0371$) were lower for pupils living in urban area than those living in rural area. Parasitemia of both parasite species were similar in single and co-infection situations ($P > 0.05$). The interaction between these parasites could involve another mechanism than a competition for blood resources. This study outlined that *M. perstans* and *P. falciparum* are co-endemic in the Akonolinga health District. In order to guarantee future success in control and eradication of malaria, a bigger attention should be given to *M. perstans* or other filarial where it is co-endemic with *P. falciparum*.

Keywords: *Mansonella perstans*, *Plasmodium falciparum*, Co-infection, Interaction, Akonolinga

1. Introduction

Mansonella perstans (Manson, 1891), causative agent of mansonellosis, is a vector-borne human filarial nematode [1]. It is transmitted by tiny blood-sucking midges of the genus *Culicoides*. It is one of the most prevalent human parasites, especially in Sub-Saharan Africa, where more than 14 million people are infected [2]. In high-prevalence countries such as Cameroon, mansonellosis mainly affects low-income populations living in rural areas [3]. Both *M. perstans* and other tissue-invasive helminth parasites have been shown to induce an immunosuppressed environment in the host through the production of an immunomodulatory cytokine IL-10 [4, 5]. IL-10 is well known for its ability to induce the secretion of IFN-gamma and the activation of monocytes/macrophages, thereby down regulate T-helper 1 type immune response [6]. This immunomodulation makes the host vulnerable to infectious agents (whose immune response is dependent on the Th1-type cell activity) such as *Plasmodium falciparum* [7, 8].

Plasmodium falciparum (Welch, 1896) is the highly pathogenic and most deadly parasite causing malaria in humans [9]. It is transmitted to humans by the bite of infected female mosquitoes of the genus *Anopheles*. Despite

the efforts of the international community, malaria remains the major cause of morbidity and mortality worldwide. According to the World Health Organization (WHO), in 2016, there were an estimated 216 million cases of malaria, an increase of about 5 million cases over 2015. More than 81% of malaria cases and 90% of deaths occur in Sub-Saharan Africa. This situation can be justified by the fact that *P. falciparum*, the most dangerous species among human Plasmodia and responsible for the deadly form of the disease, predominates in this part of the world [10]. In Sub-Saharan Africa, the distribution of *P. falciparum* overlaps that of several other human parasites such as *M. perstans* [2].

In this area in particular, climatic and environmental conditions as well as poverty favor the development and persistence of several parasites species and the multiparasitism become the rule rather than the exception [11]. It is recognized that, parasites that occur concomitantly within the same host individual interact either directly in the exploitation of the host limited resources or indirectly via the host's immune system [12]. These interactions may influence the severity of the disease and the dynamics of transmission of infection with implications for the epidemiology of associated diseases [13, 14]. Thus, the knowledge of interaction mechanisms between co-infecting parasites species is necessary to understand the epidemiology of the infection and the diseases caused. The present study aimed at contributing to the knowledge of interaction mechanisms between *M. perstans* and *P. falciparum*, both human hemoparasites. Thus, we projected to i) determine the prevalence of single infections with *M. perstans* and *P. falciparum* and their co-infection, ii) identify the determinants of infection with both parasites and iii) determine the possible mechanism through which these parasites interact with each other.

2. Material and Methods

2.1. Study Area

A cross-sectional study was conducted from September 2017 to July 2018 in the Akonolinga Health District, located in the Nyong et Mfoumou Division, in the Centre Region of Cameroon. Akonolinga is situated at 130 km from Yaoundé, and its Health District between 3°31'- 4°20' North latitudes and 11°55' - 12°30' East longitudes and covers a surface of 4,300 km^2 approximately with a bit more than 100,000 inhabitants [15]. The climate is typically equatorial with four discontinuous seasons. The annual average rainfall is 2,000 mm with an annual average temperature of 24°C [16]. The population, predominantly rural, essentially practices agricultural and forestry activities.

Five (05) Government schools were randomly selected: three in rural area (Essang-Ndibi, Kpwele, and Eboa) and two in urban area (Loum and Ecole publique annexe (EPA)).

2.2. Ethical Considerations

Ethical clearance and administrative authorization were obtained from the National Ethical Committee of Research for Human Health and the Yaoundé University Teaching Hospital respectively. Visits were made to the head teachers and teachers of the schools to explain the objectives and potential benefits of the study before giving out consent forms to pupils. The purpose and methodology of the survey were explained and arrangements with the teachers of each school were made on when to collect the samples. Informed consent forms were distributed to the children to take from parents/legal guardians their consent before sample collection.

2.3. Collection of Blood Samples

Before sample collection, demographic data such as name, age and sex of each pupil were documented in a structured questionnaire. After sterilizing the finger tip, 3 drops of blood were collected by pricking the finger. Two thick blood films were prepared from standardized 50 μl finger pricked blood using 75 μl non-heparinised capillary tubes. Smears were prepared by spreading the blood on clean slides covering an area of 1.5 × 2.5 cm and allowed to air-dry calibrated thick, and one thin blood film was prepared [17]. Air-dried slides were stored in slide boxes then transported to the laboratory of Parasitology, Mycology and Parasitic Immunology of the Yaoundé University Teaching Hospital for parasitological analyzis.

2.4. Sample Analysis

Research and identification of *M. perstans* and *P. falciparum*: Detection and identification of blood stages of both parasites (microfilaria for *M. perstans* and trophozoites or gametocytes for *P. falciparum*) in peripheral blood were made by ordinary microscopy using routine Giemsa stained calibrated thick and thin blood films. Prepared thick films on the field were dehaemoglobinized using tap water for 10 min, fixed with methanol for 1 min, stained in 10% Giemsa for 30 min, rinsed in tap water and allowed to air-dry. Thin smears were stained in 10% Giemsa for 30 min after fixation in methanol for 1 min [18]. Slides were read at a magnification of x200 then under immersion (x1000) in a blind manner by two well-trained technicians using CyScope® microscope (Partec-Sysmex GmbH, Görlitz, Germany). Parasites were identified using identification keys available in the laboratory. *Mansonella perstans* microfilaria count was expressed as microfilariae per milliliter of blood (μF/ml). The density of *P. falciparum* was evaluated according to a formula specific of the laboratory and expressed as trophozoites per microliter of blood (T/μl). In case of discrepancy, a third well-trained technician was called to read the quarreled slides. Slides were negative in case of absence of any trophozoite after examination of at least 10 fields for *P. falciparum* or absence of microfilaria in entire slide for *M. perstans*.

2.5. Data Analysis

Statistical analyses were performed using the software SPSS 16.0 (SPSS, Chicago, Inc., IL, USA). Frequencies (%) of socio-demographic data (age group and gender) of pupils and of the presence of *M. perstans* and *P. falciparum* in case of monoparasitism and multiparasitism were determined. Chi-squared and Fisher's exact tests were used to compare proportions. The one-way ANOVA test was used to compare the main parasites densities. Adjusted odd ratios (aOR) and 95% confidence intervals were computed to assess the association between parasites species and other variables. Significance was set at $P < 0.05$.

3. Results

3.1. Description of the Study Population

During this study, a total of 416 pupils were recruited (209 in rural area and 207 in urban area). The age varied from 4 to 15 years old. The mean age of pupils was 9.17 ± 0.27 years with 0.8 as a male-to-female sex-ratio. As presented in Table 1, gender-related distribution did not vary between schools ($P = 0.12$). In the contrary most pupils ($P<0.001$) were aged 8-11 years, 62.50% in Kpwele, 56.25% in Eboa and 42.25% in EPA schools.

Table 1. General characteristics of the study population.

| | Total population (N = 416) | Rural area (n = 209) | | | Urban area (n=207) | | P |
		Essang-Ndibi (n=73)	Kpwele (n=72)	Eboa (n=64)	EPA (n=76)	Loum (n=131)	
Gender %(n)							
Male	45.67 (190)	45.21 (33)	50.00 (36)	35.94 (23)	38.16 (29)	52.67 (69)	0.122
Female	54.33 (226)	54.79 (40)	50.00 (36)	64.06 (41)	61.84 (47)	47.33 (62)	
Age group (years) %(n)							
4 – 7	32.45 (135)	41.09 (30)	26.39 (19)	28.12 (18)	32.89 (25)	32.82 (43)	
8 – 11	40.63 (169)	34.25 (25)	62.50 (45)	56.25 (36)	42.11 (32)	23.67 (31)	<0.001
12 – 15	26.92 (112)	24.66 (18)	11.11 (8)	15.63 (10)	25.00 (19)	43.51 (57)	

N: Total number of pupils, n: number of pupils in the school;%: Frequency.

3.2. Prevalence of Single Parasite Species and Co-infection

Out of the 416 blood samples examined, 251 (60.34%) were negative, 157 (37.74%) harbored only one of both parasites (10 children harbored *M. perstans* alone while 147 pupils were only infected by *P. falciparum*), and 8 (1.92%) were co-infected by *M. perstans* and *P. falciparum*.

The prevalence of *M. perstans* and *P. falciparum* in the analyzed population were 4.32% (18 pupils infected) (95%CI= 2.76 - 6.74) and 37.26% (155 pupils infected) (95%CI = 32.75 - 42.00) respectively. The mean parasite densities were 508.7 ± 310.3 (min: 430, max: 1300) µF/ml of blood for *M. perstans* and 5240.38 ± 2037.42 (min: 857, max:

10400) T/µl of blood for *P. falciparum*. It should be noted that, microfilariae of *Loa loa* were also found among 2 (0.48%) children.

The prevalence of *M. perstans* ranged between 2.29% (Loum) and 8.34% (Kpwele) but did not statistically differ between these schools ($P = 0.20$). Conversely, the prevalence of *P. falciparum* significantly varied ($P = 0.006$) between schools with the lowest value in Loum (28.24%) and the highest one in Eboa (56.24%) (Table 2). The rate of the co-infection with both parasites was significant ($P = 0.034$) higher in school from rural area (3.35%) compared to that of urban counterpart (0.48%).

Table 2. Prevalence of single infections and co-infection in different schools.

Area	Schools (Frequency)	Single infection *M. perstans* n (%)	Single infection *P. falciparum* n (%)	Co-infection *M. perstans* and *P. falciparum* n (%)
Rural	Essang-Ndibi (73)	3 (4.11)	25 (34.24)	2 (2.74)
	Kpwele (72)	3 (4.17)	24 (33.33)	3 (4.17)
	Eboa (64)	0 (0.00)	34 (53.12)	2 (3.12)
Urban	EPA 1 (76)	2 (2.63)	28 (36.84)	0 (0.00)
	Loum (131)	2 (1.53)	36 (27.48)	1 (0.76)
	Total (416)	10 (2.40)	147 (35.33)	8 (1.92)

n: number of cases,%: prevalence

3.3. Factors Associated with the Infections

Results of logistic regression analysis outlined the significant influence of living area on the risk of either single infections or co-infection (Table 3). Indeed, pupils living in the urban area were lesser at risk for single infections (aOR = 0.46; 95%CI: 0.12-0.78; P = 0.0264 and aOR = 0.64; 95%CI:

0.42-0.99; P = 0.0432) for *M. perstans* and *P. falciparum* respectively and co-infection (aOR = 0.10; 95%CI: 0.01-0.87; P = 0.0371). Besides, both age and gender of participants did not significantly influence the single infections or co-infection risk (P > 0.05) as summarized in Table 3.

Table 3. *Factors associated with single infections and co-infection with M. perstans and P. falciparum.*

Variables	Single infection *M. perstans* aOR [95%CI]	P	Single infection *P. falciparum* aOR [95%CI]	P	Co-infection *M. perstans* and *P. falciparum* aOR [95%CI]	P
Gender						
Male	1		1		1	
Female	0.32 [0.08 – 1.28]	0.1066	0.90 [0.60 – 1.37]	0.6291	0.42 [0.10 – 1.85]	0.2531
Age group (years)						
4 - 7	1		1		1	
8 – 11	0.61 [0.13 - 2.88]	0.5351	1.58 [0.97 – 2.59]	0.0688	0.73 [0.14 – 3.77]	0.7023
12 – 15	1.04 [0.22 – 4.95]	0.9621	1.41 [0.81 – 2.45]	0.2273	1.19 [0.19 – 7.65]	0.8509
Area						
Rural	1		1		1	
Urban	0.46 [0.12 – 0.78]	0.0264*	0.64 [0.42 – 0.99]	0.0432*	0.10 [0.01 – 0.87]	0.0371*

aOR: Adjusted Odds Ratio; 95%CI: Confidence interval at 95%; *P: P*-value; *: Significant

3.4. Association Between M. perstans and P. falciparum Infection

Co-infection with *M. perstans* and *P. falciparum* was less frequent than under the assumption of the independence ($\chi2$ = 0.41, P = 0.51) indicating no statistical association between both parasites. Mean parasite density of *P. falciparum* did not vary significantly (P = 0.890) between co-infection with *M. perstans* (4908.8 ± 1569.17 T/µl) compared to mono-infection (5582.4±2524.13 T/µl). Likewise, the mean microfilariemia of *M. perstans* did not vary significantly (P = 0.36) between co-infection with *P. falciparum* (609.2±301.2 µF/ml) and mono-infection (592.9 ±290.7 µF/ml).

4. Discussion

This study showed the co-endemicity of *P. falciparum* and *M. perstans* in the Akonolinga Health District with a high frequency of *P. falciparum* but low frequencies of *M. perstans* and co-infection of both parasites. In general, many parasite species are endemic in tropical regions of the world and in sub-Saharan Africa in particular, where multiparasitism appears as the rule rather than the exception. Therefore, new approaches to easily detect and recognize multiple infections should be developed in this part of the world. Actually, the most exciting challenge for researchers, specific for disease ecologists is moving from "one parasite species - one host" system towards an ecosystem view "multiple parasites species - multiple hosts", embracing the real complexity of natural systems [19]. Understanding parasitic interactions within-host and landscape level is necessary for good knowledge of the transmission and pathogenesis of diseases caused, and for developing more effective strategies to fight against parasitic diseases [20].

Mansonellosis is considered as a neglected tropical disease (NTD) because it afflicts the poorest people, those without access to safe water, sanitation and basic health services required in order to protect themselves against infections. Mansonellosis has not been linked with a clear and distinct clinical picture [2]. The prevalence of *M. perstans* obtained in this study is lower than those obtained by Tatuene *et al*. [21] 14.2% in the same region and by Wanji *et al*. [18] 70.0% and Drame *et al*. [22] 76% in other areas of Cameroon.

Conversely, it is higher than that found by M'bondoukwé *et al*. [23] 1.1% in Gabon. These discrepancies may be related mainly to difference in study design and area. These authors worked on the general population while this study focused on children. In endemic areas, it is thought that the prevalence and density of *M. perstans* microfilaraemia increase gradually with age, and reach highest levels in the adult individuals [2].

In this work, the prevalence was independent of gender and age groups but was dependent on living area. The prevalence of *M. perstans* was highest in rural area; this finding is in conformity with other reports by Bassene *et al*. [24] in Senegal and Debrah *et al*. [25] in Ghana. Indeed, it is known that high prevalence of *M. perstans* occurs mainly in areas where tropical forests alternate with large swamps and open ground [2]. This can explain why we obtain high prevalence in rural area.

The rate of the co-infection *M. perstans* - *P. falciparum* was lower in this study than those found by Tatuene *et al*. [21] 5.1% in general population in the same region and Hillier *et al*. [26] 2.29% among pregnant women in Uganda and by Drame *et al*. [22] 15.1% in the general population in eastern Cameroon. Differences can be explained by the different study population and diagnostic techniques. Molecular biology technique used by these authors is more sensitive than the microscopy technique used in this study.

It was assumed the existence of a competition for resource exploitation between *M. perstans* and *P. falciparum*, given that both parasite species share the same ecological niche in humans, namely the circulatory system. But no statistical association and no significant difference in parasitemia of these single infections and co-infection situations were found, thereby outlining the absence of any negative interaction between these pathogens agents. This finding is consistent with that of Fokom-Domgue *et al*. [27]. The interaction between these parasites could involve a mechanism other than a competition for blood resources. However, Metenou *et al*. [8] found that the presence of *M. perstans* was impairing the magnitude and quality of cell-mediated immunity to malaria parasites through depletion in CD4+ T cells and a complete absence of multifunctional Th1 cells. More investigations should be done to clearly

understand how these parasites interact.

5. Conclusion

The present study outlined that *M. perstans* and *P. falciparum* are co-endemic in the Akonolinga Health district with a high prevalence of *P. falciparum* but low frequencies of *M. perstans* and co-infection of both parasites. The study also outlined the influence of living area on the risk of single and co-infection and the absence of competitive interaction between *M. perstans* and *P. falciparum*. However, despite the fact that, this study outlined the absence of any negative interaction between these pathogens agents that share same ecological niche in human, we suggest a multidisciplinary study involving mathematicians that will be helpful for further analysis of interaction mechanisms between *P. falciparum* and *M. perstans* in co-endemic areas. In order to guarantee success in control and eradication of malaria in Africa, a bigger attention should be given to areas where *M. perstans* or other filarial species and *P. falciparum* are co-endemic.

Conflict of Interest

The authors declare having no competing interests regarding this publication.

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