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Multiple filarial species microfilaraemia: a comparative study of areas with endemic and sporadic onchocerciasis

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ABSTRACT

Background & objectives: The study was aimed at determining the pattern of co-occurrence of species of microfilaraemia between onchocerciasis endemic and sporadic populations.

Methods: From every consenting person of one year and above, 50 µl of day and night blood samples were collected and processed respectively with Haematoxylin and Giemsa as vital stains. Two skin snips (one each from the waist and the shoulder) were also taken from these individuals and processed.

Results: Results showed single species microfilaraemia (86.4 and 82.3%), double species microfilaraemia (12.2 and 16.9%) and triple species microfilaraemia (1.4 and 0.7%) for endemic and sporadic populations respectively. All the species had single species microfilaraemia mostly, but *Mansonella perstans* and *Loa loa* showed greatest tendency towards double and triple species microfilaraemia. The prevalence of *Wuchereria bancrofti* microfilaraemia among those positive for *Onchocerca volvulus* was significantly lower than the overall prevalence of *Wuchereria bancrofti*. *Wuchereria bancrofti* microfilaraemia was most common among those who had *L. loa* microfilaraemia. *Wuchereria bancrofti* microfilarial intensity was higher among those with *M. perstans* microfilaraemia than among those positive for any of the other filarial species. Similarly, the intensity of *M. perstans* microfilaraemia among those positive for *W. bancrofti* exceeded the overall intensity of *M. perstans*.

Conclusion: It is concluded that there was no definite pattern in mf densities discernible from co-occurrence infections either in the onchocerciasis endemic or sporadic population. There could be varied outcomes of onchocerciasis infection attributable to positive or negative regulatory effects of other pathogens harbored by the victims.

Key words Co-infections; endemic; filarial species; microfilaraemia; Nigeria; onchocerciasis

INTRODUCTION

Five species of filarial parasites have been reported in Nigeria, namely *Wuchereria bancrofti*, *Onchocerca volvulus*, *Loa loa*, *Mansonella perstans* and *M. streptocerca*^{1,2}. Studies on these infections have been carried in most parts of Nigeria, including south-eastern parts of the country³⁻¹². These studies have shown that these infections are widespread, and that the prevalence of microfilaraemia and clinical manifestations vary from one locality to another in south-eastern Nigeria, except for *M. streptocerca*.

In the Imo River Basin, both endemic onchocerciasis (EO) and sporadic onchocerciasis (SO) have been reported in the Upper and Lower parts respectively^{4,5,8}. The EO area is known to support the breeding of 11 species of blackflies including the *Simulium damnosum* complex, which is the main vector of endemic onchocerciasis in south-eastern Nigeria¹³. In addition, bancroftian filariasis, mansonellosis and loiasis are all widespread and endemic in both parts of the basin^{3,9-11}.

There have been few reports of co-infections of filarial worms in literature most of which have focused on the diagnostic difficulties of parasitic co-infections¹⁴, while others have bothered on the contribution of bacteria to inflammatory disease pathogenesis and the use of antibiotic therapy as a novel method of treatment¹⁵⁻¹⁸.

Studies on co-infections involving the four human filarial infections have not yet been undertaken. This study is therefore aimed at determining the prevalence and patterns of multiple filarial species infections between EO and SO populations in the Imo River Basin, Nigeria.

MATERIAL & METHODS

Study area and population

The study was conducted in two parts of the Imo River Basin. Endemic onchocerciasis is found in the Upper IRB⁵, an area with undulating plains and fast flowing streams. Southerly and closer to the shore is the Lower IRB, an area with relatively slow flowing rivers, swamps and sporadic onchocerciasis⁸. Farming is the main occupation in

both parts of the IRB, but fishing is also a major occupation in the Lower IRB. Fish-smoking, a local traditional way of preserving fish is a regular practice among the older women.

Blood sampling and examination

From every consenting person of one year and above, 50 µl of day and night blood samples were collected and processed respectively with Haemotoxylin and Giemsa as vital stains. Detailed description of the collection and processing day and night blood samples for parasitological examination has been reported¹⁰. Identification was according to the keys in Learning Bench Aid No. 3 (Tropical Health Technology).

Skin snipping

Two skin snips (one each from the shoulder and the buttocks) for parasitological examination were taken from each individual during day time using a Walser corneal punch. Processing for microscopy was as described elsewhere^{5,8}.

Ethical approval

Ethical clearance was given by the Ministry of Health of both Okigwe and Emohua Local Government Areas, whose field staff actually mobilized the community and assured them of our compliance with ethical standards.

RESULTS

General observations

The occurrence of *W. bancrofti*, *O. volvulus*, *M. perstans*, and *L. loa* microfilaraemia separately, or together in double or multi-species microfilaraemia in both the EO and SO populations was analyzed. A total of 500 individuals were positive for microfilaraemia of at least one of these four filarial species in the EO area, while it was 419 individuals in the SO area. Individuals who were 10 yr old and above constituted 91.8% of microfilaraemic cases in EO area as against 94.5% in the SO area (Table 1).

In the EO area, 432 (84.6%) had single species microfilaraemia; 61 (12.2%) had double species microfilaraemia; while 7 (1.4%) had triple species microfilaraemia. Similarly, in the SO area, 345 (82.3%) had single species microfilaraemia; 71 (16.9%) had double species microfilaraemia; while three (0.7%) had triple species microfilaraemia. The major difference observed between the two populations was that those positive for the three categories of infection (single, double and triple) were dominated by those in the 10+ yr age group in the SO population

Table 1. Comparison of selected parameters between onchocerciasis endemic and sporadic areas of Imo River Basin, Nigeria

| Category | Endemic population | Sporadic population | Remarks ($p = 0.05$) |
|-----------------------------|--------------------|---------------------|------------------------|
| 1-9 yr | 41 (8.2) | 23 (5.5) | |
| 10+ yr | 459 (91.8) | 396 (94.5) | Not significant |
| Single infection (all ages) | 432 (86.4) | 345 (82.3) | Not significant |
| Double infection (all ages) | 61 (12.2) | 71 (16.9) | |
| Triple infection (all ages) | 7 (1.4) | 3 (0.7) | |

Figures in parentheses indicate percentages.

while in the EO population, those under 10 yr of age featured almost equally in the single and double species microfilaraemia categories (Fig. 1). There were no cases of quadruple species microfilaraemia in both the populations. The occurrence of the four microfilarial species as single, double and triple infections in both the populations was analyzed (Table 2). In the EO population, single species microfilaraemia constituted most of the microfilaraemic cases as follows: *O. volvulus* (83.9%), *W. bancrofti* (79.1%), *L. loa* (53.6%), and *M. perstans* (51.6%). Double species microfilaraemia was dominated by *M. perstans* (43.7%), whereas in triple species microfi-

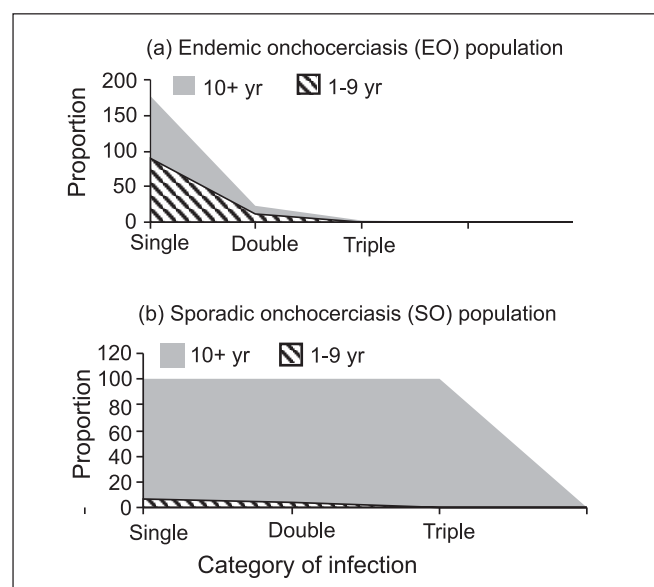


Fig. 1: Relative proportions of single, double, and triple species microfilaraemia in (a) endemic, and (b) sporadic onchocerciasis populations. In the EO population, the proportions of those positive in the single, double and triple infections were comparable in both age categories, while those above 10 (10+) yr predominated the three categories of infection in the SO population.

Table 2. The occurrence of single, double and triple infection with microfilarial species in the endemic and sporadic areas of the Imo River Basin

| Species | No. examined | Total (+)ve | No. (+)ve (% total +ve) | | |
|---------------------|--------------|-------------|-------------------------|------------------|------------------|
| | | | Single infection | Double infection | Triple infection |
| <i>W. bancrofti</i> | 1010* | 43 | 34 (79.1) | 6 (14) | 3 (7) |
| | 1486† | 100 | 75 (75) | 22 (22) | 3 (3) |
| <i>O. volvulus</i> | 1024* | 379 | 318 (83.9) | 55 (14.5) | 5 (1.3) |
| | 1525† | 54 | 46 (85.2) | 7 (13) | 0 (0) |
| <i>M. perstans</i> | 1010* | 126 | 65 (51.6) | 54 (43.7) | 7 (5.6) |
| | 1486† | 166 | 110 (66.3) | 60 (36.1) | 3 (1.8) |
| <i>L. loa</i> | 1024* | 28 | 15 (53.6) | 7 (25) | 6 (21.4) |
| | 1525† | 178 | 114 (64) | 53 (29.8) | 3 (1.7) |

*EO area; †SO area; Figures in parentheses indicate percentages.

laraemia *L. loa* (21.4%) presented the highest occurrence. Similarly, in the sporadic onchocerciasis study population, single species microfilaraemia presented were as follows: *O. volvulus* (85.2%), *W. bancrofti* (75%), *M. perstans* (66.3%) and *L. loa* (64%). *L. loa* (29.8%) occurred most in double species microfilaraemia, whereas it was *M. perstans* (1.8%) for triple species microfilaraemia.

Relationships between the filarial species

In the following analyses of relationships between the different microfilarial species, only individuals of 10 yr and above were included, because microfilaraemia among children below 10 yr of age was not common.

Cluster I: Co-infections among those positive for *W. bancrofti* microfilaraemia

The co-occurrence between *W. bancrofti* microfilaraemia and microfilaraemia due to other human filarial species in the EO population was calculated (Table 3) and the overall prevalence of *W. bancrofti* microfilaraemia was 5.1%. The overall prevalence of *W. bancrofti* microfila-

raemia was lower than the prevalence of *W. bancrofti* among those who had *L. loa* microfilaraemia (3.6%), *M. perstans* microfilaraemia (2.7%), or *O. volvulus* microfilaraemia (0.3%). Due to low number of microfilaraemic individuals, these differences were not analyzed statistically. The equivalent analyses for the SO area showed that overall prevalence of *W. bancrofti* microfilaraemia was 7.7%.

The *W. bancrofti* mf prevalence in SO population among those positive for *L. loa* microfilaraemia (10.1%) was the highest and was significantly higher than both the *W. bancrofti* mf prevalence among those positive for *O. volvulus* microfilaraemia (1.9%), and those positive for *M. perstans* microfilaraemia (1.9%) (χ^2 -test; $p < 0.01$, for both the tests).

In the SO population, the overall *W. bancrofti* mf GMI was 199 mf/ml, and this was significantly lower than the *W. bancrofti* mf GMI among both those who had *M. perstans* microfilaraemia (396 mf/ml) and those who had *L. loa* microfilaraemia (251 mf/ml) (t -test; $p < 0.001$ for both the tests).

Table 3. Interaction between *W. bancrofti* microfilaraemia and other microfilarial species in the endemic and sporadic onchocerciasis populations aged 10 yr and above

| Population | No. of individuals | No. with <i>W. bancrofti</i> mf | Prevalence of <i>W. bancrofti</i> mf (%) | Mf GMI (mf/ml)* |
|---------------------------------------|--------------------|---------------------------------|--|-----------------|
| Total population | 802** | 41 | 5.1 | 132 |
| | 1224† | 93 | 7.7 | 199 |
| Population with <i>O. volvulus</i> mf | 350** | 1 | 0.3 | – |
| | 54† | 1 | 1.9 | – |
| Population with <i>M. perstans</i> mf | 112** | 3 | 2.7 | 565 |
| | 156† | 4 | 1.9 | 396 |
| Population with <i>L. loa</i> mf | 28** | 1 | 3.6 | – |
| | 169† | 17 | 10.1 | 251 |

*Only indicated if there are ≥ 3 mf positive individuals; **Endemic onchocerciasis area; †Sporadic onchocerciasis area.

Table 4. Relationship between *O. volvulus* microfilaraemia and other microfilarial species in the onchocerciasis endemic and sporadic populations aged 10 yr and above

| Population | No. of individuals | No. with <i>O. volvulus</i> mf | Prevalence of <i>O. volvulus</i> mf (%) | Mf GMI (mf/ml)* |
|--|--------------------|--------------------------------|---|-----------------|
| Total population | 815** | 350 | 42.9 | 16 |
| | 1249† | 54 | 4.3 | 22 |
| Population with <i>W. bancrofti</i> mf | 41** | 1 | 2.4 | – |
| | 93† | 1 | 1.1 | – |
| Population with <i>M. perstans</i> mf | 112** | 49 | 41.1 | 15 |
| | 156† | 6 | 3.8 | 21 |
| Population with <i>L. loa</i> mf | 28** | 5 | 17.9 | 25 |
| | 169† | 0 | 0 | 0 |

*Only indicated if there are ≥ 3 mf positive individuals; **Endemic onchocerciasis area; †Sporadic onchocerciasis area.

Cluster II: Co-infection among those positive for *O. volvulus* microfilaraemia

Interaction between *O. volvulus* microfilaraemia and other microfilarial species in the EO and SO populations was assessed. The overall prevalence of *O. volvulus* microfilaraemia was 42.9% (Table 4). In this cluster in the EO, the prevalence of *O. volvulus* microfilaraemia was comparable among those positive for *M. perstans* microfilaraemia (41.1%; χ^2 -test; $p > 0.05$), but significantly lower among those who had *L. loa* microfilaraemia and *W. bancrofti* microfilaraemia (17.9 and 2.4%, respectively; χ^2 -test; $p < 0.01$ for both tests). The overall *O. volvulus* mf GMI was 16 mf/snip, which was comparable with the *O. volvulus* mf GMI among those who had *M. perstans* microfilaraemia (15 mf/snip; t -test; $p > 0.05$), but was significantly higher than *O. volvulus* mf GMI among individuals who had *L. loa* microfilaraemia (25 mf/snip; t -test; $p < 0.001$).

In the SO population, the overall prevalence of *O. volvulus* microfilaraemia was 4.3%, and was comparable

to the prevalence of *O. volvulus* microfilaraemia among those positive for *M. perstans* (3.8%) (χ^2 -test; $p > 0.05$), but significantly lower among those (in the cluster) positive for *W. bancrofti* microfilaraemia (1.1%) (χ^2 -test; $p < 0.05$). In this cluster, the overall mf GMI was 22 mf/snip, which is comparable to *O. volvulus* mf GMI among individuals positive for *M. perstans* microfilaraemia (21 mf/snip; t -test; $p > 0.05$).

Cluster III: Co-infections among those positive for *M. perstans* microfilaraemia

The overall prevalence of *M. perstans* microfilaraemia in the EO area was 14% (Table 5). This was comparable to the prevalence of *M. perstans* microfilaraemia among those who had *O. volvulus* microfilaraemia (13.1%), but considerably lower among those who had *W. bancrofti* microfilaraemia (7.3%) and *L. loa* microfilaraemia (3.6%). The overall *M. perstans* mf GMI was 105 mf/ml, which was significantly higher among those positive for *W. bancrofti* microfilaraemia (184 mf/ml) and *O. volvulus*

Table 5. Relationship between *M. perstans* microfilaraemia and other microfilarial species in the endemic and sporadic populations aged 10 yr and above

| Population | No. of individuals | No. with <i>M. perstans</i> mf | Prevalence of <i>M. perstans</i> mf (%) | Mf GMI (mf/ml)* |
|--|--------------------|--------------------------------|---|-----------------|
| Total population | 802** | 112 | 14 | 105 |
| | 1224† | 156 | 12.7 | 121 |
| Population with <i>W. bancrofti</i> mf | 41** | 3 | 7.3 | 184 |
| | 93† | 3 | 3.2 | 460 |
| Population with <i>O. volvulus</i> mf | 350** | 46 | 13.1 | 132 |
| | 54† | 6 | 11.1 | 192 |
| Population with <i>L. loa</i> mf | 28** | 1 | 3.6 | – |
| | 169† | 41 | 24.3 | 80 |

*Only indicated if there are ≥ 3 mf positive individuals; **Endemic onchocerciasis area; †Sporadic onchocerciasis area.

Table 6. Relationship between *L. loa* microfilaraemia and other microfilarial species in the endemic and sporadic onchocerciasis populations aged 10 yr and above

| Population | No. of individuals | No. with <i>L. loa</i> mf | Prevalence of <i>L. loa</i> mf (%) | Mf GMI (mf/ml)* |
|--|--------------------|---------------------------|------------------------------------|-----------------|
| Total population | 815** 1249† | 28 169 | 3.4 13.5 | 231 154 |
| Population with <i>W. bancrofti</i> mf | 41** 93† | 1 17 | 2.4 18.3 | – 183 |
| Population with <i>O. volvulus</i> mf | 350** 54† | 5 0 | 1.4 0 | 665 – |
| Population with <i>M. perstans</i> mf | 112** 156† | 1 41 | 0.9 26.3 | – 114 |

*Only indicated if there are ≥ 3 mf positive individuals; **Endemic onchocerciasis area; †Sporadic onchocerciasis area.

microfilaraemia (132 mf/ml) (*t*-test; $p < 0.05$ for both the tests).

In the SO area, the overall mf prevalence of *M. perstans* was 12.7%, and was significantly lower than the prevalence of *M. perstans* microfilaraemia among those positive for *L. loa* microfilaraemia (24.3%; χ^2 -test; $p < 0.001$); comparable to that of those positive for *O. volvulus* microfilaraemia (11.1%; χ^2 -test; $p > 0.05$), but significantly higher than the prevalence of *M. perstans* microfilaraemia among those positive for *W. bancrofti* microfilaraemia (3.2%; χ^2 -test; $p < 0.01$). The overall *M. perstans* mf GMI was 121 mf/ml, which was significantly higher than the *M. perstans* mf GMI of among those positive for *W. bancrofti* microfilaraemia (460 mf/ml), and that among those positive for *O. volvulus* microfilaraemia (192 mf/ml) (*t*-test; $p < 0.05$ for both tests), but significantly lower than the *M. perstans* mf GMI among those positive for *L. loa* microfilaraemia (80 mf/ml) (*t*-test; $p < 0.05$).

Cluster IV: Co-infection among those positive for *L. loa* microfilaraemia

The overall prevalence of *L. loa* in the EO area was 3.4% (Table 6), and this was lower than the prevalence of *L. loa* microfilaraemia among those positive *W. bancrofti* microfilaraemia (2.4%), *O. volvulus* microfilaraemia (1.4%) and *M. perstans* microfilaraemia (0.9%). These differences were not analyzed statistically due to low number of microfilaraemic individuals in these groups. The overall *L. loa* mf GMI was 231 mf/ml and was significantly lower than the *L. loa* mf GMI among those who had *O. volvulus* microfilaraemia (665 mf/ml; *t*-test; $p < 0.001$).

In the SO population, the overall prevalence of *L. loa* microfilaraemia was 13.5%. This was significantly lower than the prevalence of *L. loa* microfilaraemia among individuals positive for *M. perstans* microfilaraemia (26.3%; χ^2 -test; $p < 0.05$), and but comparable to that of those posi-

tive for *W. bancrofti* microfilaraemia (18.3%) (χ^2 -test; $p > 0.05$). There was no positive case of *L. loa* microfilaraemia among individuals positive for *O. volvulus* microfilaraemia in the sporadic onchocerciasis population. The overall *L. loa* mf GMI was 154 mf/ml, which was significantly lower than the *L. loa* mf GMI among those who had *W. bancrofti* microfilaraemia (183 mf/ml; *t*-test; $p < 0.001$), but higher than that among those positive for *M. perstans* microfilaraemia (114 mf/ml; *t*-test; $p < 0.05$).

Triple species co-infections

The triple filarial infections were not statistically analyzed because the number of cases in the sporadic onchocerciasis area was small.

DISCUSSION

Prevalence of single, double and triple species filarial infection

Wuchereria bancrofti, *M. perstans*, and *L. Loa* are endemic in all the parts of the Imo River Basin. Endemic onchocerciasis is restricted to the Upper Imo River Basin regarded as EO area in this study. The proportions of microfilaraemia of different categorizations in both the EO and SO populations were to large extent comparable and similar to reports of filarial species co-infections reported elsewhere¹⁹.

Of all the four filarial species examined in this study, *O. volvulus* and *W. bancrofti* exhibited greatest tendencies towards single species microfilaraemia in both the study populations, whereas *M. perstans* and *L. loa* showed greatest proclivity towards double and triple species microfilaraemia in both EO and SO areas. This is suggestive of relatively higher probability of concomitant infection involving *M. perstans* and *L. loa* than involving *O. volvulus* and *W. bancrofti* in either populations.

Possible case of antagonistic interaction

The difference between the overall prevalence of *W. bancrofti* and the prevalence of *W. bancrofti* microfilaraemia among those positive for *O. volvulus* was statistically significant in both the study populations. Similarly, the prevalence of *O. volvulus* microfilaraemia among those who were positive for *W. bancrofti* microfilaraemia was not only lower than the overall prevalence, but was also lowest of the prevalences among those positive for mf microfilaraemia of any other human filarial species in both the study populations. There is need for further studies to ascertain if there is possible heterologous antagonistic interaction between the two microfilarial species.

Multi-parasitism is a common feature in tropical countries^{19,20} and many examples of heterologous interaction among other parasitic infections have been reported. Studies on interactions between different filarial species and other parasite species in arthropod vectors have shown that secondary infection with *B. pahangi* microfilariae by intrathoracic inoculation reduced the development rate of a pre-existing *Plasmodium gallinaceum* infection, both in susceptible and refractory strains of *Ae. aegypti*²¹. Co-infections of helminth and *P. falciparum* infections have been shown to have clinical importance²². Interestingly, co-infection with *Trypanosoma cruzi* protects mice from early death and *P. berghei* co-infected mice survived longer, symptoms of cerebral malaria were absent, and breakdown of brain blood barrier was less pronounced in mice co-infected with *T. cruzi*²³. The intensity of *P. falciparum* infection tends to be reduced in children with concomitant infection with measles or influenza, while infection with *Bordetella pertussis* was found not to influence the intensity of *P. falciparum* infection. It has been observed that *W. bancrofti* and *P. falciparum* influenced each other in a given host during concomitant infection¹⁹, and this was probably because of induction of inflammatory cytokines during both malaria infection²⁴, and lymphatic filariasis infection²⁵. Such cytokines may affect the course of both helminth and protozoa infections²⁶. With concomitant microfilaraemia, *P. falciparum* followed a more benign course in monkeys²⁷ and susceptibility to cerebral malaria was down-regulated in mice simultaneously infected with the filarial parasite *Brugia pahangi*²⁸. It is evident from these studies that cytokine production induced by *W. bancrofti* may play an important role in its interaction with other parasites, such as *O. volvulus*.

Co-infection with combinations of helminths and malaria parasites are reportedly common. Such infections may have considerable health consequences, leading to more severe clinical symptoms and pathology than for infection with single parasite species²⁹. They further concluded that

interaction of malaria and helminth infections increases the severity of anaemia and organomegaly observed in schoolchildren and thus may potentially create a great challenge for disease control in the tropics. Some findings suggest that co-infection with multiple parasites could alter the immune responses³⁰.

Some evidence from experimental models point to the possible existence of heterologous interaction. All who had triple species microfilaraemia in this study were >10 yr old, while most of the infected children had single species microfilarial infection. This is consistent with the observed age-related patterns of filarial infection with prevalence increasing with age. The period between first exposure and first appearance of microfilariae in the blood is between five and eight years for *W. bancrofti*^{31–34}. The probability for multi-species filarial infection in children is therefore minimal.

Different stages of filarial worms stimulate different Th-cell subsets³⁵. The microfilariae of *O. volvulus* and *W. bancrofti* have different surface characteristics, and it is known that microfilariae show higher levels of AChE activity than adult worms^{36,37}. Since antigens of *O. volvulus* microfilariae can induce non-specific suppressor cells activity *in vitro*³⁸, we can surmise that the larval stages could be the most important phase for study on any possible heterologous interaction between *O. volvulus* and *W. bancrofti*.

Cases of no definite interaction

Wuchereria bancrofti microfilaraemia was the most common among those who had *L. loa* microfilaraemia in both the EO and SO populations, but due to small sample sizes, definite conclusions could not be drawn on whether there exists any synergistic interaction between these two filarial species. However, there was a higher *W. bancrofti* microfilarial intensity among those with *M. perstans* microfilaraemia than among those positive for any of the other filarial species in both the study populations. Similarly, the intensity of *M. perstans* microfilaraemia among those positive for *W. bancrofti* exceeded the overall intensity of *M. perstans* in both the study populations at statistically significant levels. The existence of any synergistic interaction between *W. bancrofti* and *M. perstans* is a subject for further studies.

In both the study populations, the prevalence and intensity of *O. volvulus* microfilaraemia among those positive for *M. perstans* microfilaraemia were comparable to the overall levels of *O. volvulus* microfilaraemia. It is interesting to note that the prevalence of *M. perstans* microfilaraemia among those positive for *O. volvulus* was in the same range as the overall prevalence of *M. perstans*

microfilaraemia in both the study populations. This may be indicative of a possible lack of interaction between *O. volvulus* and *M. perstans*. This requires verification using immunological methods.

The prevalence of *M. perstans* microfilaraemia among the cluster positive for *L. loa* was significantly lower than the overall prevalence of *M. perstans* microfilaraemia in the onchocerciasis endemic population, but significantly higher than the overall prevalence of *M. perstans* microfilaraemia in the sporadic onchocerciasis population. *Loa loa* and *M. perstans* are the most widespread and ubiquitous of all the human filarial species in Nigeria, most times occurring in the same areas. It can be inferred that perhaps, negative interaction does not exist between the two species. The common concomitant infections between the two species could partly be attributed to their transmission ecologies which are sympatric. The vectors of both the infections bite mostly outdoors in the forest. However, the pertinent questions, which constitute the kernel of a planned future study are: why are concomitant infections between the two species significantly low in EO area but significantly higher in SO area? Does onchocerciasis endemicity antagonize concomitant infection involving *L. loa* and *M. perstans*? As of now, what is known is that antigens of *O. volvulus* microfilariae can induce non-specific suppressor cells activity *in vitro*³⁸.

The overall prevalence of *L. loa* microfilaraemia was significantly lower than the prevalence of *L. loa* among those positive for *W. bancrofti* microfilaraemia in the EO population, but in the SO population, both the prevalences were comparable. But what could explain these? There are many questions than there are answers.

The pertinent question to be asked is “Since co-infection with multiple strains may reduce the total amount of parasite (‘parasitic load’) in an infected person, will parasitic load increase if a person is infected with only two strains instead of all four?”³⁹. It might be necessary to add: was mf intensity observed to be significantly reduced in this study? The answer is that there was no evidence of a definite pattern of mf density established in this study whether in the EO or SO populations.

In conclusion, we can learn from concomitant infections involving non-filarial patients, where a different thread of observations has been reported. We can draw inference from the fact that in most regions where onchocerciasis is endemic, there could be “varied outcomes of onchocerciasis infection attributable to positive or negative regulatory effects of other pathogens harboured by the patients”²³. Further studies are underway to unravel patterns of relationships between these human filarial infections in onchocerciasis endemic and sporadic populations.

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