

Co-infection with *Onchocerca volvulus* and *Loa loa* microfilariae in central Cameroon: are these two species interacting?

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SUMMARY

Ivermectin treatment may induce severe adverse reactions in some individuals heavily infected with *Loa loa*. This hampers the implementation of mass ivermectin treatment against onchocerciasis in areas where *Onchocerca volvulus* and *L. loa* are co-endemic. In order to identify factors, including co-infections, which may explain the presence of high *L. loa* microfilaraemia in some individuals, we analysed data collected in 19 villages of central Cameroon. Two standardized skin snips and 30 µl of blood were obtained from each of 3190 participants and the microfilarial (mf) loads of both *O. volvulus* and *L. loa* were quantified. The data were analysed using multivariate hierarchical models. Individual-level variables were: age, sex, mf presence, and mf load; village-related variables included the endemicity levels for each infection. The two species show a certain degree of ecological separation in the study area. However, for a given individual host, the presence of microfilariae of one species was positively associated with the presence of microfilariae of the other (OR = 1.79, 95% CI [1.43–2.24]). Among individuals harbouring *Loa* microfilariae, there was a slight positive relationship between the *L. loa* and *O. volvulus* mf loads which corresponded to an 11% increase in *L. loa* mf load per 100 *O. volvulus* microfilariae. Co-infection with *O. volvulus* is not sufficient to explain the very high *L. loa* mf loads harboured by some individuals.

Key words: *Onchocerca volvulus*, *Loa loa*, microfilariae, co-infection, association, Cameroon.

INTRODUCTION

The filarial nematode *Loa loa* is a parasite of humans and several simian species that is found only in Central Africa. The microfilariae of the human strain show a diurnal periodicity in the peripheral blood, and the parasite is mostly transmitted by 2 ombrophilic tabanids: *Chrysops dimidiata* and *C. silacea*. The geographical distribution of *L. loa* is closely related to the distribution of its vectors and the prevalences of infection are particularly high in forested regions. However, high prevalences of loiasis have been reported from savanna areas of north Cameroon and of other countries (Boussinesq and Gardon, 1997; Kamgno and Boussinesq, 2001).

Clinical signs of human loiasis are usually mild (e.g. Calabar swellings, subconjunctival migration of adult worms) though severe complications such as

nephropathy (Katner, Beyt and Krotoski, 1984; Pakasa, Nseka and Nyimi, 1997), and spontaneous encephalopathy (Kivits, 1952; Cauchie *et al.* 1965; Samé Ekobo *et al.* 1981) have also been associated with *L. loa* infection. In some parts of Africa, loiasis has been reported as a major health issue for the afflicted populations (Boulesteix and Carme, 1986), but concerns about loiasis have increased with the implementation, in forest areas, of large-scale ivermectin distribution programmes to control onchocerciasis. Ivermectin treatment may induce severe adverse reactions, including fatal encephalopathy, in individuals harbouring high *L. loa* microfilarial (mf) loads (Gardon *et al.* 1997; Boussinesq *et al.* 2003). Thus, in areas where *L. loa* and *Onchocerca volvulus* are co-endemic, the risk of post-ivermectin severe adverse events (SAEs) hampers the implementation of the African Programme for Onchocerciasis Control (APOC). Specific measures have been taken to reinforce the surveillance of SAEs during ivermectin distribution in communities where the prevalence of *L. loa* microfilaraemia is >20%. In these communities, more than 1% of the population

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may harbour more than 30 000 microfilariae (mfs) per ml of blood and thus have an increased risk of neurological SAEs (Boussinesq *et al.* 2001).

At present, the within-individual processes influencing the mf status of a given host, and the immunological mechanisms regulating the *L. loa* mf loads are not completely understood. It has recently been shown that, in addition to a genetic predisposition to becoming microfilaraemic (Garcia *et al.* 1999), a high exposure to incoming infective larvae might play an essential role in the appearance of *L. loa* microfilaraemia (Pion *et al.* 2005). A detailed analysis of age- and sex-specific parasitological profiles of loiasis has shown that the mean mf loads among microfilaraemic individuals were fairly constant among age groups (Pion *et al.* 2004).

Increasing empirical evidence supports the notion that host infection with one helminth species can influence the outcome of infection with other helminths (Haswell-Elkins, Elkins and Anderson, 1987; Booth *et al.* 1998; Behnke *et al.* 2001; Cox, 2001; Howard, Donnelly and Chan, 2001; Lello *et al.* 2004; Faulkner *et al.* 2005), with recent theoretical studies investigating the possible nature of such interactions (Bottomley, Isham and Basáñez, 2005). Though human co-infection by *L. loa* and *O. volvulus* raises important public health concern, to our knowledge, the association at individual host level between these two species has not been adequately assessed.

In order to identify whether the co-infection with *O. volvulus* might explain the presence of high *L. loa* microfilaraemia in some individuals, we used statistical models to analyse the association between *L. loa* microfilaraemia and *O. volvulus* microfilaridermia. We based our analysis on data collected prior to the implementation of large-scale ivermectin treatment in a population living in central Cameroon, the area where most of the severe, and sometimes fatal, post-ivermectin adverse reactions have been reported (Twum-Danso, 2003).

PATIENTS AND METHODS

Study area, selection of subjects and parasitological examinations

The data analysed in the present paper were collected as part of epidemiological surveys of filarial infections, conducted in 1991–1993 in the Lékié Division of the Central Province of Cameroon. During these surveys, a total of 3244 consenting individuals aged ≥ 5 years (1447 males and 1797 females) were examined in 19 villages that had been selected after stratification according to the distance between their location and the Sanaga River. The Lékié Division is an area of degraded forest located on the left bank of the loop of the Sanaga River, near its confluence with the Mbam River. A number of sites presenting a

series of rapids constitute particularly productive breeding sites for the *Simulium* species transmitting onchocerciasis in this area of Cameroon.

Diagnosis of Loa loa infection

A blood sample was collected from each individual by finger-prick, between 10:00 and 16:00 h, in a non-heparinized capillary tube, and calibrated thick blood films (1 per individual) were immediately prepared, using 30 μl of blood. Each Giemsa-stained smear was then examined under a low-power microscope and all the *L. loa* mfs present on the slide were counted.

Diagnosis of Onchocerca volvulus infection

Examinations were done using the methods recommended by the Onchocerciasis Control Programme in West Africa, OCP (Moreau, Prost and Prod'hon, 1978; Prost and Prod'hon, 1978): 2 skin snips (one from each iliac crest) were taken using a 2 mm Holth-type corneoscleral punch in 16 of the villages and a 1.5 mm punch in the remaining 3 villages, and left to incubate in normal saline for 24 h. The emerging mfs were counted and the individual mf loads were calculated as the arithmetic mean of the number of mfs/skin snip (mfs/ss). To account for the difference of size of the snips taken with the 1.5 mm and 2 mm Holth punches, the former were multiplied by 1.33.

All the participants were registered and information on their age, sex and date of any previous treatment with antifilarial therapy was recorded. Data from 54 individuals (18 males and 36 females) who had been treated during the last 5 years were discarded from the analyses presented here.

Statistical analysis

We investigated the associations between *L. loa* and *O. volvulus* in the human host using regression models to adjust the observed associations for exogenous individual- and community-level factors, that is, factors independently associated with the presence and density of mfs of one or the other species. Infection with *O. volvulus* has already been shown to be influenced by individual-level characteristics such as host sex and age (Basáñez and Boussinesq, 1999; Pion *et al.* 2004). At the community level, the degree of endemicity for *L. loa* and *O. volvulus* (measured in terms of mf prevalence or intensity), and the density of their respective vectors (measured in terms of biting rates), vary following distinct geographical and ecological factors (e.g. distance from the village to the breeding sites for onchocerciasis).

To account for ecological factors possibly acting at the community level, we defined endemicity

variables for each filarial infection. For onchocerciasis, the level of endemicity was defined by the Community Microfilarial Load (CMFL), i.e. the geometric mean of the mf densities among the population (both sexes) aged ≥ 20 years (Remme *et al.* 1986). For loiasis, and following Boussinesq *et al.* (2001), the level of endemicity was defined according to the prevalence of microfilaraemia among adults aged ≥ 15 years (PMF). These prevalence values were standardized for age and sex using the OCP standard population (Moreau *et al.* 1978) modified for its application to a population ≥ 15 years old. We defined 3 classes of endemicity for each species: for onchocerciasis, we considered CMFLs <15, 15–49·9 and ≥ 50 mfs/ss and, for loiasis, PMFs <20%, 20–27·9% and $\geq 28\%$. These classes were chosen to represent the range of infection intensity and prevalence among villages while ensuring sufficient and similar numbers in each group.

The analysis was divided into 2 sections. Section 1 considered the *L. loa* microfilaraemia as a qualitative (presence/absence) outcome, and looked for factors associated with the presence or absence of *L. loa* mfs among individuals. Section 2 focused on quantitative association (mf loads) between the two species. We used the software package MLwiN (Rasbush *et al.* 2004) to fit multilevel (or hierarchical) logistic regression models. Multilevel models are an extension of standard regression models that use random effects to allow for unobserved factors at the community-level (or at higher clustering levels), in addition to observed individual- and community-level factors (Goldstein, 2003).

For the qualitative analysis, we used the logistic model (model A) to assess whether the presence/absence of *L. loa* microfilaraemia was associated with the presence of *O. volvulus* microfilaridermia. Two sets of estimates were obtained for model A. The first set contains the adjusted estimate of the effect of *O. volvulus*, where adjustments were made for individual-level (age and sex, where the former was treated as a continuous variable) and community-level factors (model A_a). The second set contains 'interaction' estimates of the effect of *O. volvulus* (model A_i). 'Interaction' refers to the regression interactions between two or more variables, and should not be confused with the within-host/biological interaction between *L. loa* and *O. volvulus*. Model A_i is an extension of model A_a, to allow separate *O. volvulus* effects for communities with the different levels of loiasis and onchocerciasis endemicity. There are 6 estimates of the effect of *O. volvulus* from model A_i, one for each of *L. loa*-*O. volvulus* endemicity combinations within which the sample villages were observed: (I) low *L. loa* and low *O. volvulus*; (II) medium *L. loa* and low *O. volvulus*; (III) high *L. loa* and low *O. volvulus*; (IV) low *L. loa* and medium *O. volvulus*; (V) medium *L. loa* and medium *O. volvulus*, and (VI) low *L. loa* and high *O. volvulus*

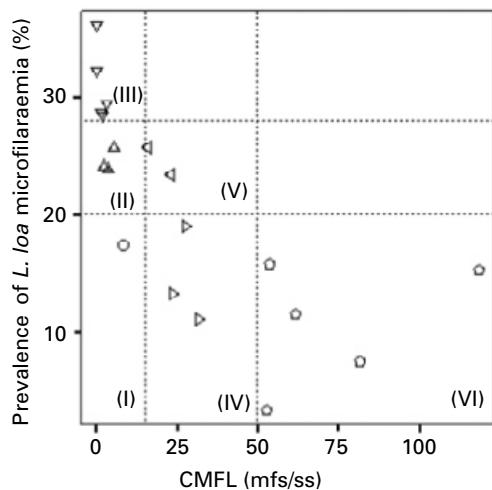


Fig. 1. Distribution of the 19 villages surveyed in Central Cameroon according to their respective levels of endemicity for *Loa loa* and *Onchocerca volvulus* microfilarial infection: (I) low *L. loa* and low *O. volvulus*; (II) medium *L. loa* and low *O. volvulus*; (III) high *L. loa* and low *O. volvulus*; (IV) low *L. loa* and medium *O. volvulus*; (V) medium *L. loa* and medium *O. volvulus*, and (VI) low *L. loa* and high *O. volvulus*.

(see Fig. 1). The total numbers of patients examined in each of these 6 situations can be found in Table 1.

For the quantitative analysis, we looked for factors associated with the patients' *L. loa* mf load, and, in the first instance, we assessed the relationship between the *L. loa* and *O. volvulus* mf loads using non-parametric Spearman's correlations (r_s). As in previous studies (Basáñez *et al.* 1999; Pion *et al.* 2004), we found evidence of high overdispersion (with variance to mean ratio values ranging between 170 and 1800, see Table 1) in the distributions of both *L. loa* and *O. volvulus*. This motivated the use of negative binomial regression models. However, the sample had an excess of zero *L. loa* loads compared to the best fitting negative binomial distribution, because only 60% of the population are genetically predisposed to be microfilaraemic for *L. loa* (Garcia *et al.* 1999). Hence, as the negative binomial distribution fitted closely the distribution of the *L. loa* positive loads (with mean = 377·4; $k = 0\cdot26$; χ^2 goodness of fit test: $P < 0\cdot31$), we excluded zero loads and fitted negative binomial models to those positive *L. loa* loads only. Alternatively, we could have used a zero-truncated negative binomial model here, but such models cannot be fitted in MLwiN. We assessed whether this would have made a difference to our results by comparing the results from fitting negative binomial regression using MLwiN without random effects to those from a truncated negative binomial model using STATA. The resulting differences between estimates were small and did not affect the interpretation of the models. Therefore, and for the sake of consistency we used MLwiN throughout.

Table 1. Arithmetic mean (AM), standard deviation (s.d.), and variance to mean ratio (VMR) for the distribution of microfilarial loads of *O. volvulus* and *L. loa* among hosts in the 6 groups of villages defined according to endemicity levels: (I) low *L. loa* and low *O. volvulus*; (II) medium *L. loa* and low *O. volvulus*; (III) high *L. loa* and low *O. volvulus*; (IV) low *L. loa* and medium *O. volvulus*; (V) medium *L. loa* and medium *O. volvulus*, and (VI) low *L. loa* and high *O. volvulus*

Group of villages	(I)		(II)		(III)		(IV)		(V)		(VI)	
	Mf species	O. v.	<i>L. loa</i>	O. v.	<i>L. loa</i>	O. v.	<i>L. loa</i>	O. v.	<i>L. loa</i>	O. v.	<i>L. loa</i>	O. v.
AM*	72.76	34.15	21.47	115.11	8.89	155.72	94.14	37.57	82.60	72.54	146.54	28.28
s.D.	202.38	172.87	60.62	365.87	46.93	514.40	130.08	146.18	141.45	360.53	155.94	150.46
VMR	562.95	875.08	171.16	1162.90	247.63	1700.25	179.73	568.77	242.22	1791.86	165.94	800.50
Maximum mf load*	1584	2061	497	3509	827	6477	688	1374	651	5257	678	1725
No. of individuals	318		503		865		514		286		704	

* Expressed as the number of mfs/skin snips for *O. volvulus* and the number of mfs/30 µl blood for *L. loa*.

As with the qualitative analysis, ‘adjusted’ (B_a) and ‘interaction’ (B_i) estimates were obtained for the negative binomial model. This analysis provides relative risk estimates, whose interpretation in our context, is more precisely the increase in *L. loa* mf load per unit increase in *O. volvulus* mf load. Therefore we use the term relative load (RL) to refer to the parameters we obtained. Thus, for quantitative variables (e.g. *O. volvulus* mf load, age) RL is the ratio of mean *L. loa* load when explanatory factor is $[x+1]$ to mean *L. loa* load when the explanatory factor is $[x]$ (holding all other factors constant). For qualitative variables (e.g. sex, *L. loa* endemicity) RL is the ratio of mean *L. loa* load for that category compared to the baseline category.

RESULTS

General information

Among the 3190 individuals examined in 19 villages, the overall prevalence of *O. volvulus* microfilaridermia was 65.8% (respectively 69.8% and 64.7% in *L. loa* positive and *L. loa* negative individuals). The overall prevalence of *L. loa* microfilaraemia was 21.9% (respectively 23.2% and 19.4% in *O. volvulus* positive and *O. volvulus* negative individuals). Some 15.3% of the individuals presented mfs of both species.

The levels of endemicity for each infection in the different villages are presented in Fig. 1. Amongst the 19 villages, the CMFL and the PMF were negatively and highly significantly correlated ($r_S = -0.895$, $P < 10^{-5}$, $n = 19$).

Qualitative analysis

A visual inspection of the *L. loa* prevalence age profiles suggested that for a given level of *L. loa* endemicity, such age profiles did not appear to be affected by the level for onchocerciasis endemicity (Fig. 2). Nonetheless, according to model A_a , the association between the presence of *L. loa* and the presence of *O. volvulus* was positive and significant ($OR = 1.790$, 95% CI [1.431–2.238]). In addition, the presence of *L. loa* microfilaraemia was dependent on age and sex of individuals. When including interactions to allow the effect of *O. volvulus* to vary between communities of different endemicity class (model A_i), none of the estimates of association between the presence of *L. loa* and the presence of *O. volvulus* in the 6 endemicity groups were significant (for example in group I, $OR = 2.111$ (95% CI [0.938–4.751]). These results also indicate that there is insufficient evidence to reject the hypothesis that the effect of *O. volvulus* on *L. loa* does not vary by endemicity group (Table 2).

The variance of the random effect distribution was very small in both the adjusted and interaction

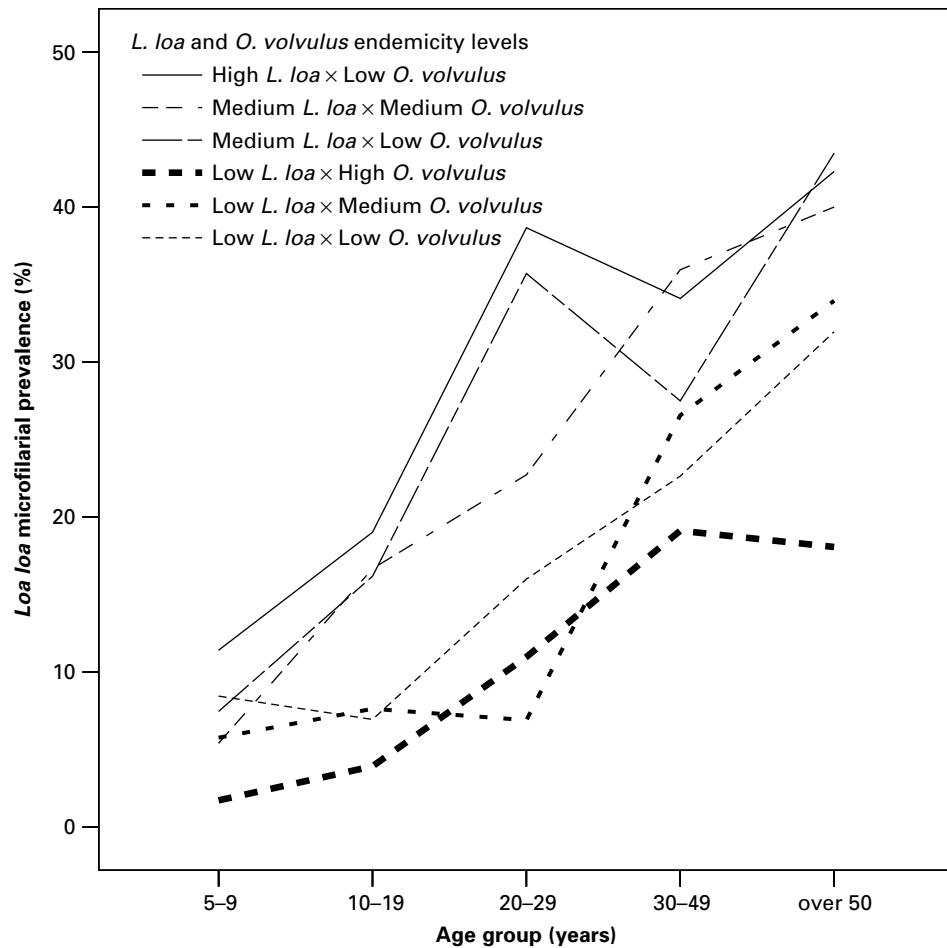


Fig. 2. Age profiles of *Loa loa* microfilarial prevalence in the different endemicity groups stratified according to the 3 *L. loa* endemicity levels and to the 3 onchocerciasis endemicity levels.

models (results not shown), indicating that much of the unobserved variation between villages had been accounted for by observed individual- and community-level factors. Thus, at this stage of the analysis, our results for model A indicate that (a) model A_a supports the hypothesis for a positive association between the presence of *Loa* microfilaraemia and *O. volvulus* infection and (b) there is insufficient evidence to support model A_i over model A_a.

Quantitative analysis

The L shape relationship observed between the individual *L. loa* and *O. volvulus* mf loads (Fig. 3) suggested a possible negative correlation between the loads. Using the log ($x+1$) transformation, with x being the microfilarial load (including zero counts), the correlation between the mf loads was positive but not significant ($r_S=0.020$, $P=0.255$, $n=3190$). However, when grouping these individuals in their villages, classifying villages according to their respective endemicity levels for the two parasite species and considering the three following age-groups, 5–19, 20–49 and ≥ 50 y, we found significant positive

correlations between the loads in those individuals: those aged 5–19 living in endemicity groups III or IV, those aged 20–49 living in endemicity group III, and those aged ≥ 50 living in endemicity groups I, II, III or VI (Fig. 4).

The negative binomial regression results are shown in Table 3 and confirm the trend observed in the analysis of correlations. In the adjusted model (B_a) the association between the positive *L. loa* loads and the *O. volvulus* loads (including zero counts) was positive and significant, but the strength of this association was small when interpreted as a relative increase of *L. loa* mf load per *O. volvulus* microfilaria. However, the relative load of 1.001 (95% CI [1.000–1.002]) equates to an 11% increase in mean *L. loa* load for every 100 mfs/ss increase in *O. volvulus* load. (The increase from [$x+100$] to [x] is $(1.001)^{100} = 1.11$ or an 11% relative load.) Similarly to the qualitative analysis, the results from the interaction model (B_i) did not support the hypothesis that the effect of *O. volvulus* on *L. loa* varies by village endemicity level. In other words, the association between the mf loads was not specific to villages with particular levels of endemicity for one or the other infection.

Table 2. Estimates (odd ratios and 95% confidence intervals) obtained by hierarchical logistic models to assess the association between the presence of *L. loa* microfilarial infection and *O. volvulus* microfilarial infection (qualitative analysis)

(Two models were assessed: adjusted on individual factors and endemicity levels in the communities (A_a), and including interaction terms between endemicity levels and *O. volvulus* infection (A_i).)

Variable	Category	Model (A_a) – Adjusted		Model (A_i) – with interaction terms	
		OR	95% CI	OR	95% CI
<i>O. volvulus</i> individual microfilaridermia	Absence	1		1	
	Presence	1.790	1.431–2.238	2.111	0.938–4.751
Sex	Female	1		1	
	Male	1.624	1.354–1.949	1.629	1.358–1.955
Age		1.075	1.054–1.096	1.082	1.061–1.104
	Age squared (Age^2)	0.999	0.999–1.000	0.999	0.999–1.000
<i>O. volvulus</i> endemicity	Low	1		1	
	Med	0.889	0.665–1.188	0.492	0.119–2.036
	High	0.482	0.329–0.705	0.461	0.113–1.880
<i>L. loa</i> endemicity	Low	1		1	
	Med	1.835	1.373–2.452	2.106	0.902–4.922
	High	2.385	1.699–3.347	2.710	1.221–6.018
Interaction: <i>O. volvulus</i> endemicity × <i>L. loa</i> endemicity	All others			1	
	Med × Med			3.421	0.682–17.169
Interaction: <i>O. volvulus</i> endemicity × <i>O. volvulus</i> individual microfilaridermia	All others			1	
	Med × Presence			1.808	0.420–7.785
	High × Presence			1.018	0.239–4.342
Interaction: <i>L. loa</i> endemicity × <i>O. volvulus</i> individual microfilaridermia	All others			1	
	Med × Presence			0.834	0.333–2.092
	High × Presence			0.855	0.358–2.041
Interaction: <i>O. volvulus</i> endemicity × <i>L. loa</i> endemicity × <i>O. volvulus</i>	All others			1	
	Med × Med × Presence			0.300	0.055–1.620
Constant		0.099	0.071–0.139	0.087	0.040–0.188

Note: In this table, ‘interaction’ refers to the regression interactions between two or more variables, and should not be confused with the within-host/biological interaction between the two parasite species.

DISCUSSION

It is well known that mixed parasitic infections in general, and helminth infections in particular, are the rule rather than the exception in both human and non-human hosts (Christensen *et al.* 1987; Cox, 2001). Concerning filariases, the results of previous studies suggest the existence of a positive association between *O. volvulus* and *Wuchereria bancrofti* (Keita *et al.* 1981; Engelbrecht *et al.* 2003), and between *O. volvulus* and *Mansonella perstans* (Gbary *et al.* 1987). However, little is known about the association between *O. volvulus* and *L. loa* mf infections despite the medical importance of this association with respect to microfilaricidal treatment. To address this point, we have explored a large dataset obtained from an area of Cameroon known to be co-endemic for loiasis and onchocerciasis, and where many post-ivermectin serious adverse reactions have been reported (Twum-Danso, 2003).

At the time of surveys in 1991–1993, neither mass treatment with ivermectin or any other filaricidal drug nor any vector control had been implemented in the area. When some of the villages included in the present study were again visited in 1995–1996 (just before commencement of the first large-scale ivermectin distribution), the levels of *L. loa* mf infections were very similar to the ones recorded during the first surveys (Boussinesq *et al.* 2001); it is the results of the former surveys that are analysed in the present study. We can thus assume that both parasite populations were at endemic equilibrium among the host populations prior to control-induced perturbations.

At the village level, we found a negative correlation between the prevalence of *L. loa* in those aged ≥ 15 y and the *O. volvulus* community microfilarial load (in those aged ≥ 20 y). Despite the fact that the study area is endemic for both species, none of the villages presented high endemicity levels for *L. loa* and

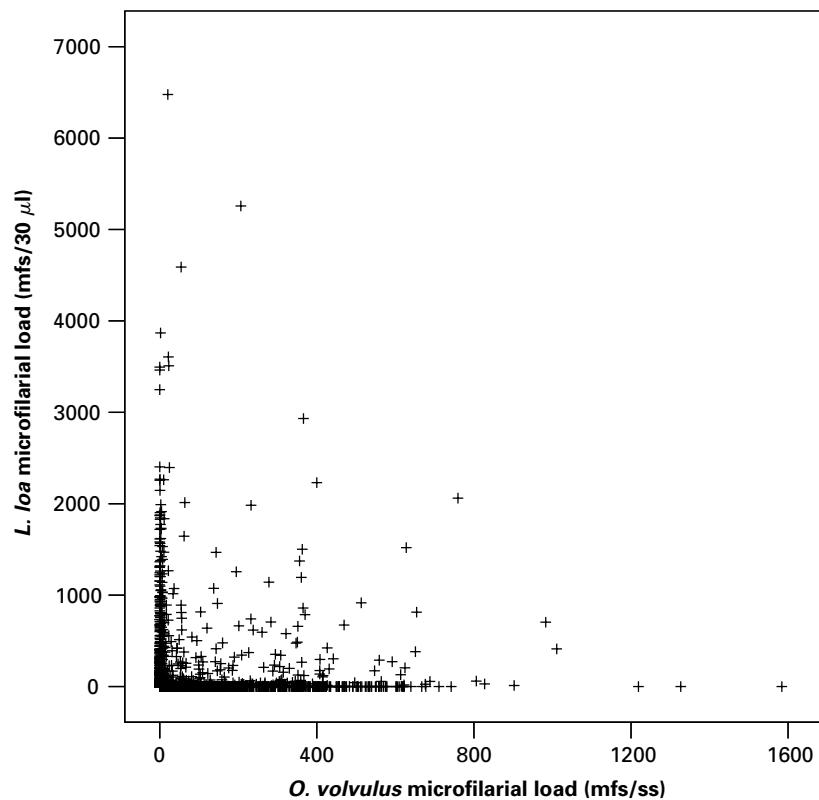


Fig. 3. Scatter plot of *Loa loa* and *Onchocerca volvulus* microfilarial loads amongst the 3190 individuals examined in the Lékié area (Central Cameroon).

medium or high endemicity levels for onchocerciasis, neither did they exhibit medium endemicity levels for *L. loa* and high endemicity level for onchocerciasis.

This apparent antagonistic correlation between the two infections might be explained by ecological factors influencing the respective geographical distributions of the vectors that transmit the two filarias in the area: the densities of the *Simulium* vectors decrease gradually from the breeding sites in the Sanaga River towards the more distant forest areas, whereas the densities of *Chrysops* are probably lower nearest the Sanaga River, where plant cover is scarcer than in others parts of the Lékié area. As said in the results section, the variance of the random effect in models (A_a) and (A_i) was very small, indicating that the observed individual- and community-level factors considered in the analysis had already accounted for much of the unobserved variation between villages. Our results highlight the importance of adjusting not only for individual factors, but also for ecological factors, when assessing the strength and direction of an association between two parasite species.

The results of the 'adjusted' and 'interactions' logistic models confirmed that the presence of a *L. loa* microfilaraemia increases with age and is more frequent in the male than in the female population, as previously reported (Pion *et al.* 2004).

After adjusting for age and sex of individuals and for ecological variation between villages (model A_a),

we found that the probability of harbouring *L. loa* mfs was significantly positively associated with the presence of *O. volvulus* microfilaridermia. The inclusion of interaction terms in the models makes the effect of *O. volvulus* on *L. loa* borderline non-significant within each of the endemicity groups (model A_i). The total numbers of individuals in each of the 6 village groups (I to VI) were fairly high, but it remains a possibility that the application of model A_i to a larger sample would have demonstrated clear-cut significant differences in the effect of *O. volvulus* on the presence of *L. loa* microfilaraemia by village endemicity level.

Considering all the individuals with positive *L. loa* loads, and after appropriate adjustment (models B_a and B_i), we found a slight but significant positive association between the positive *L. loa* loads and *O. volvulus* mf loads, these latter including zero counts. Again, these results underline that use of unadjusted correlation might lead to misleading trends.

Taken together, our results suggest that, overall in the studied human population, there is a trend towards a positive association between the presence of *L. loa* and of *O. volvulus* microfilariae and that, among people harbouring *L. loa* mfs, there is also a positive, yet slight association between the two species. The present findings differ from the results obtained in the southwest province of Cameroon by Wanji *et al.* (2003). In this latter study, no significant association was found between *L. loa* and *O. volvulus*

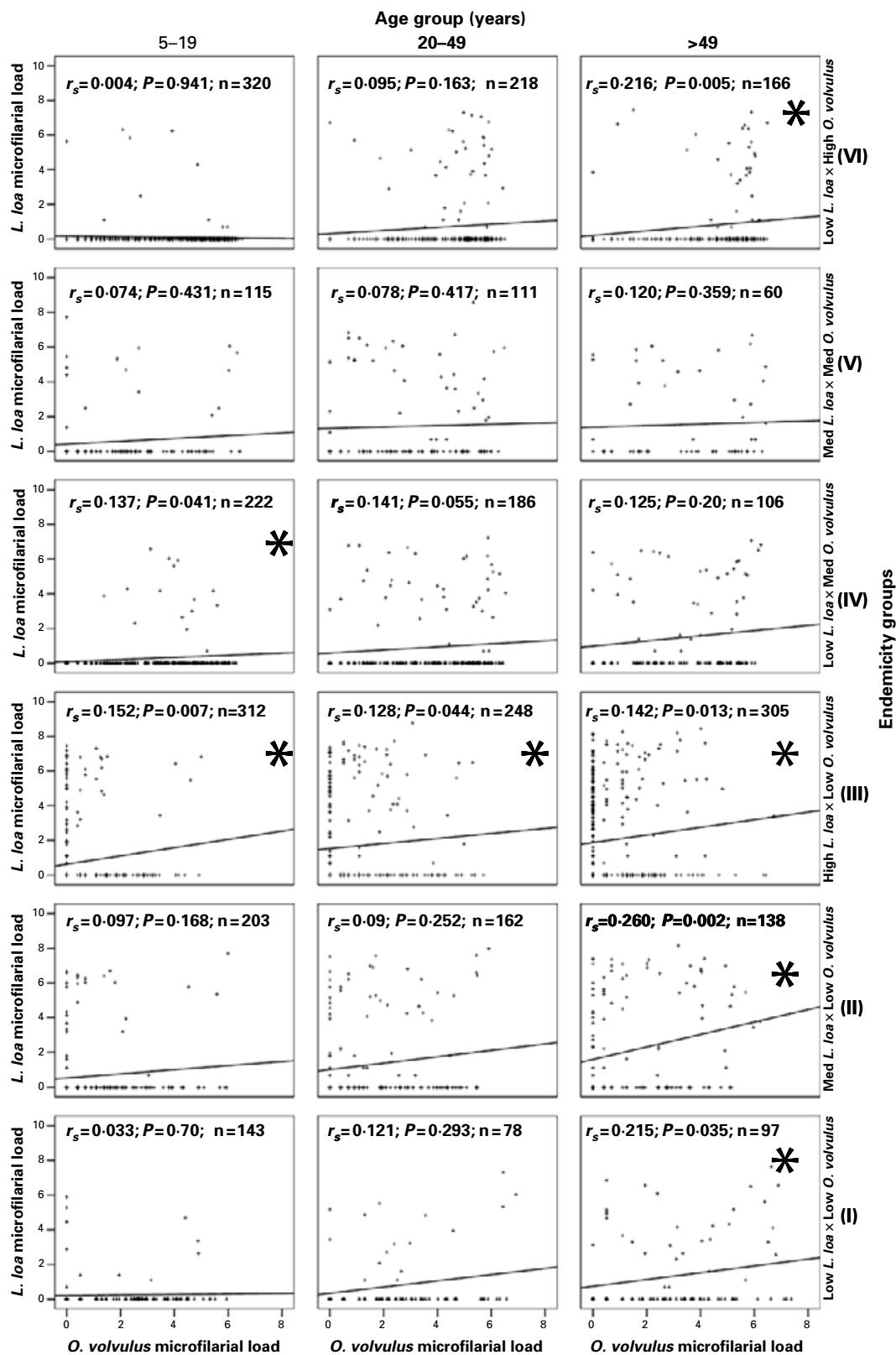


Fig. 4. Scatter plots of *Loa loa* and *Onchocerca volvulus* microfilarial loads showing the relationship between the loads in different age- and endemicity-groups. The loads are represented as log ($x + 1$) and expressed as number of microfilariae per skin snip for *O. volvulus* and as number of microfilariae per 30 μl blood for *L. loa*. The presence of a star in the graph indicates a significant correlation between the loads. The lines represent least squares estimates and, although error structure is clearly not normal, they are shown to help the reader judge the general direction of the trend. Estimates obtained with the more appropriate negative binomial regression were very similar (not shown).

Table 3. Estimates (relative loads* and 95% confidence intervals) of the hierarchical negative binomial models to assess the association between the positive *L. loa* microfilarial (mf) loads and the positive or negative *O. volvulus* mf loads (quantitative analysis)

(Two models were assessed: adjusted on individual factors and endemicity levels in the communities (B_a) and including interaction terms between endemicity levels (B_i) and *O. volvulus* mf load.)

Variable	Category	Model (B_a) – Adjusted		Model (B_i) – with interaction terms	
		RL	95% CI	RL	95% CI
<i>O. volvulus</i> individual mf load	Continuous	1.001	1.000–1.002	1.002	1.001–1.003
Sex	Female	1		1	
	Male	0.807	0.636–1.022	0.798	0.627–1.015
Age		1.027	1.000–1.056	1.025	0.998–1.054
Age squared (Age^2)		1.000	0.999–1.000	1.000	0.999–1.000
<i>O. volvulus</i> endemicity	Low	1		1	
	Med	0.720	0.474–1.093	1.570	0.652–3.823
	High	1.015	0.550–1.875	2.098	0.838–5.250
<i>L. loa</i> endemicity	Low	1		1	
	Med	1.826	1.161–2.871	3.554	1.623–7.783
	High	2.361	1.446–3.854	3.908	1.784–8.559
Interaction:	All others			1	
<i>O. volvulus</i> endemicity				0.366	0.132–1.013
$\times L. loa$ endemicity					
Interaction:	Low			1	
<i>O. volvulus</i> endemicity				0.999	0.996–1.002
$\times O. volvulus$ mf load				0.998	0.995–1.001
Interaction:	Low			1	
<i>L. loa</i> endemicity $\times O. volvulus$				1.001	0.998–1.003
mf load				0.998	0.995–1.001
Interaction:	All others			1	
<i>O. volvulus</i> endemicity $\times L. loa$				1.002	0.995–1.002
endemicity $\times O. volvulus$ mf load					
Constant		249.885	158.894–392.981	144.604	65.528–305.137

* RL: Relative load. For quantitative variables (e.g. *O. volvulus* mf load, age) is the ratio of mean *L. loa* load when explanatory factor is $[x+1]$ to mean *L. loa* load when explanatory factor is $[x]$ (holding all other factors constant). For qualitative variables (e.g. sex, *L. loa* endemicity) the RL is the ratio of mean *L. loa* load for that category compared to the baseline category.

infection (*P*-value for χ^2 test: 0.074). However, in the study by Wanji *et al.* several factors might have led to an inaccurate estimation of the possible association between *L. loa* and *O. volvulus* mf infections. First, in the study area, the overall prevalence of *L. loa* microfilaraemia was rather low (8.6% versus 21.9% in the present study) and, on the other hand, *O. volvulus* infection had been determined by nodule palpation only, a rapid epidemiological assessment method good enough for operational purposes but clearly not suited to obtaining accurate estimates of mf prevalence. Low endemicity of loiasis and probably underestimated prevalence of onchocerciasis may have led to low statistical power when assessing the association between the two infections. Secondly, the statistical analysis, limited to qualitative aspects (absence/presence of *L. loa* mfs and *O. volvulus* nodules), was performed without any adjustment for individual factors or endemicity levels, which constitute an important source of variance between

individual co-infections, as demonstrated in the present study. These differences in methodology may explain the different conclusions drawn from the two studies.

According to Bottomley *et al.* (2005), a feature of synergistic intra- and interspecific interactions is that they frequently lead to equilibrium correlations between species that are positive in sign for younger hosts but rapidly approach zero with host age, making their detection and measurement harder than in the case of antagonistic interactions. The effects that we describe here are slight yet positive after controlling statistically for the effects of age, sex, and endemicity. Also, we do not think that these positive effects are due to positively correlated exposures that may be masking underlying antagonistic interactions, as the densities of the simuliid and tabanid vectors vary in opposing ways with distance from the Sanaga river. We therefore proceed by distinguishing between 2 possible types of interaction

mechanisms, (a) those acting directly on the parasites, and (b) those mediated through host immunity that may give rise to synergistic effects.

Concerning possible direct interactions on the one hand, the two species live in different compartments within the human body: *O. volvulus* adult worms live in subcutaneous nodules and their microfilariae are principally found in the lymphatic system, particularly in the dermal layers of the skin; by contrast, *L. loa* adults live freely in subcutaneous tissues and their microfilariae circulate in the blood. Although these differences may make a direct interaction between the microfilariae unlikely, they do not rule out the possibility that interactions may take place at other stages in the life-cycle, such as the point of establishment of incoming larvae, so that the infection by one species would facilitate infection by the other species.

On the other hand, it has been shown that filarial infections may provoke a profound immunosuppression of the host (Ottesen, Weller and Heck, 1977; Maizels and Yazdanbakhsh, 2003; Maizels *et al.* 2004) that can be parasite-specific (leading to synergistic intraspecific effects), or mediated through immunological cross-reactions, leading to interspecific effects (Wahl *et al.* 1998). In onchocerciasis, as well as in loiasis, the intensity of microfilarial infection has been found to be associated with high levels of IL-10 cytokine (Winkler *et al.* 1999; Hoerauf and Brattig, 2002) and for this reason, IL-10 cytokine has been suspected to play a key role in the immunosuppression process. Immune cross-reactions may also be cross-regulated by many different pathways and unfortunately, no experimental model supporting *O. volvulus/L. loa* co-infection is currently available for testing different hypotheses.

Besides, host immunosuppression can also be induced by components in the saliva of vectors, injected at the feeding site. It has been shown that saliva of *Simulium vittatum* affects immune cell responses and cytokine production (Cupp and Cupp, 1997). Some components of anthropophilic simuliids' saliva may also inhibit platelet aggregation, prevent coagulation and induce vasodilatation of capillaries. Repeated saliva injections by *Simulium* vectors into *Loa* infected, yet amicrofilaraemic individuals, could facilitate the appearance of *L. loa* microfilariae in the bloodstream. To test these hypotheses, studies on the effect of African *Simulium* species' saliva would be needed.

Finally, we cannot exclude the possibility that those individuals, presenting a high intensity of microfilarial infection for both species are people particularly exposed to the two infections. For instance, individuals with fishing and hunting activities might have an important contact rate with blackflies, when fishing on the banks of the river, and with *Chrysops* during daytime hunting in forested parts. Regarding onchocerciasis, this hypothesis is

supported by a recent paper on the role of exposure to vectors on the level of mf infection at the individual level (Filipe *et al.* 2005). Regarding loiasis, to our knowledge, no studies have documented the role of individual exposure on the mf load.

In conclusion, a positive qualitative association between infection with *O. volvulus* and the presence of *L. loa* microfilaremia has been found at the individual level. However, given the low strength of the quantitative association, it can be said that the two species can be considered as having separate epidemiologies. Lastly, the present study clearly demonstrated that co-infection with *O. volvulus* is not sufficient to explain why some individuals harbour high *L. loa* mf densities.

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REFERENCES

- Basáñez, M. G. and Boussinesq, M.** (1999). Population biology of human onchocerciasis. *Philosophical Transactions of the Royal Society of London (series B)* **354**, 809–826.
- Behnke, J. M., Bajer, A., Sinski, E. and Wakelin, D.** (2001). Interactions involving intestinal nematodes of rodents: experimental and field studies. *Parasitology* **122** (Suppl.), S39–S49.
- Booth, M., Bundy, D. A., Albonico, M., Chwaya, H. M., Alawi, K. S. and Savioli, L.** (1998). Associations among multiple geohelminth species infections in schoolchildren from Pemba Island. *Parasitology* **116**, 85–93.
- Bottomley, C., Isham, V. and Basáñez, M.-G.** (2005). Population biology of multispecies helminth infection: interspecific interactions and parasite distribution. *Parasitology* **131**, 417–433.
- Boulesteix, G. and Carme, B.** (1986). Encéphalite au cours du traitement de la filariose à *Loa loa* par la diéthylcarbamazine. *Bulletin de la Société de Pathologie Exotique* **79**, 649–654.
- Boussinesq, M. and Gardon, J.** (1997). Prevalences of *Loa loa* microfilaremia throughout the area endemic for the infection. *Annals of Tropical Medicine and Parasitology* **91**, 573–589.
- Boussinesq, M., Gardon, J., Gardon-Wendel, N. and Chippaux, J. P.** (2003). Clinical picture, epidemiology and outcome of *Loa*-associated serious adverse events related to mass ivermectin treatment of onchocerciasis in Cameroon. *Filaria Journal* **2** (Suppl. 1), S4.
- Boussinesq, M., Gardon, J., Kamgno, J., Pion, S. D., Gardon-Wendel, N. and Chippaux, J. P.** (2001). Relationships between the prevalence and intensity of *Loa loa* infection in the Central province of Cameroon.

- Annals of Tropical Medicine and Parasitology* **95**, 495–507.
- Cauchie, C., Rutsaert, J., Thys, O., Bonnyns, M. and Perier, O.** (1965). Encéphalite à *Loa loa*, traitée par l'association de cortisone et de carbamazine. *Revue Belge de Pathologie et de Médecine Expérimentale* **31**, 232–244.
- Cox, F. E. G.** (2001). Concomitant infections, parasites and immune responses. *Parasitology* **122** (Suppl), S23–S38.
- Christensen, N. O., Nansen, P., Fagbemi, B. O. and Monrad, J.** (1987). Heterologous antagonistic and synergistic interactions between helminths and between helminths and protozoans in concurrent experimental infection of mammalian hosts. *Parasitology Research* **73**, 387–410.
- Cupp, E. W. and Cupp, M. S.** (1997). Black fly (Diptera: Simuliidae) salivary secretions: importance in vector competence and disease. *Journal of Medical Entomology* **34**, 87–94.
- Engelbrecht, F., Oettl, T., Herter, U., Link, C., Philipp, D., Edgehore, H., Kaliraj, P. and Enwezor, F.** (2003). Analysis of *Wuchereria bancrofti* infections in a village community in northern Nigeria: increased prevalence in individuals infected with *Onchocerca volvulus*. *Parasitology International* **52**, 13–20.
- Faulkner, H., Turner, J., Behnke, J., Kamgno, J., Rowlinson, M. C., Bradley, J. E. and Boussinesq, M.** (2005). Associations between filarial and gastrointestinal nematodes. *Transactions of the Royal Society of Tropical Medicine and Hygiene* **99**, 301–312.
- Filipe, J. A. N., Boussinesq, M., Renz, A., Collins, A. C., Vivas-Martinez, S., Grillet, M.-G., Little, M. P. and Basáñez, M.-G.** (2005). Human infection patterns and heterogeneous exposure in river blindness. *Proceedings of the National Academy of Sciences, USA* **102**, 15265–15270.
- Garcia, A., Abel, L., Cot, M., Richard, P., Ranque, S., Feingold, J., Demenais, F., Boussinesq, M. and Chippaux, J. P.** (1999). Genetic epidemiology of host predisposition microfilaraemia in human loiasis. *Tropical Medicine and International Health* **4**, 565–574.
- Gardon, J., Gardon-Wendel, N., Demanga, N., Kamgno, J., Chippaux, J. P. and Boussinesq, M.** (1997). Serious reactions after mass treatment of onchocerciasis with ivermectin in an area endemic for *Loa loa* infection. *Lancet* **350**, 18–22.
- Gbary, A. R., Guiguemde, T. R., Ouedraogo, J. B. and Lechuga, P.** (1987). Etude du polyparasitisme filarien en zone de savane au Burkina Faso. *Médecine Tropicale* (Mars) **47**, 329–332.
- Goldstein, H.** (2003). *Multilevel Statistical Models* (3rd Edn). Arnold, London.
- Haswell-Elkins, M. R., Elkins, D. B. and Anderson, R. M.** (1987). Evidence for predisposition in humans to infection with *Ascaris*, hookworm, *Enterobius* and *Trichuris* in a South Indian fishing community. *Parasitology* **95**, 323–337.
- Hoerauf, A. and Brattig, N.** (2002). Resistance and susceptibility in human onchocerciasis—beyond Th1 vs. Th2. *Trends in Parasitology* **18**, 25–31.
- Howard, S. C., Donnelly, C. A. and Chan, M. S.** (2001). Methods for estimation of associations between multiple species parasite infections. *Parasitology* **122**, 233–251.
- Kamgno, J. and Boussinesq, M.** (2001). Hyperendémicité de la loase dans la plaine Tikar, région de savane arbustive du Cameroun. *Bulletin de la Société de Pathologie Exotique* **94**, 342–346.
- Katner, H., Beyt, B. E. and Krotoski, W. A.** (1984). Loiasis and renal failure. *Southern Medical Journal* **77**, 907–908.
- Keita, M. F., Prost, A., Balique, H. and Ranque, P.** (1981). Associations of filarial infections in man in the savanna zones of Mali and Upper Volta. *American Journal of Tropical Medicine and Hygiene* **30**, 590–592.
- Kivits, M.** (1952). Quatre cas d'encéphalite mortelle avec invasion du liquide céphalorachidien par *Microfilaria loa*. *Annales de la Société Belge de Médecine Tropicale* **32**, 235–242.
- Lello, J., Boag, B., Fenton, A., Stevenson, I. R. and Hudson, P. J.** (2004). Competition and mutualism among the gut helminths of a mammalian host. *Nature, London*, **428**, 840–844.
- Maizels, R. M., Balic, A., Gómez-Escobar, N., Nair, M., Taylor, M. D. and Allen, J. E.** (2004). Helminth parasites: masters of regulation. *Immunological Reviews* **201**, 89–116.
- Maizels, R. M. and Yazdanbakhsh, M.** (2003). Immune regulation by helminth parasites: cellular and molecular mechanisms. *Nature Reviews Immunology* **3**, 733–744.
- Moreau, J. P., Prost, A. and Prod'hon, J.** (1978). Essai de normalisation de la méthodologie des enquêtes clinico-parasitologiques sur l'onchocercose en Afrique de l'Ouest. *Médecine Tropicale* (Mars) **38**, 43–51.
- Ottesen, E. A., Weller, P. F. and Heck, L.** (1977). Specific cellular immune unresponsiveness in human filariasis. *Immunology* **33**, 413–421.
- Pakasa, N. M., Nseka, N. M. and Nyimi, L. M.** (1997). Secondary collapsing glomerulopathy associated with *Loa loa* filariasis. *American Journal of Kidney Diseases* **30**, 836–839.
- Pion, S. D. S., Demanou, M., Oudin, B. and Boussinesq, M.** (2005). Loiasis: the individual factors associated with the presence of microfilaraemia. *Annals of Tropical Medicine and Parasitology* **99**, 491–500.
- Pion, S. D. S., Gardon, J., Kamgno, J., Gardon-Wendel, N., Chippaux, J. P. and Boussinesq, M.** (2004). Structure of the microfilarial reservoir of *Loa loa* in the human host and its implications for monitoring the programmes of Community-Directed Treatment with Ivermectin carried out in Africa. *Parasitology* **129**, 613–626.
- Prost, A. and Prod'hon, J.** (1978). Le diagnostic parasitologique de l'onchocercose. Revue critique des méthodes en usage. *Médecine Tropicale* (Mars) **38**, 519–532.
- Rasbash, J., Steele, F., Browne, W. and Prosser, B.** (2004). *A User's Guide to MLwiN Version 2.0*. Institute of Education, London.
- Remme, J., Ba, O., Dadzie, K. Y. and Karam, M.** (1986). A force-of-infection model for onchocerciasis and its applications in the epidemiological evaluation of the Onchocerciasis Control Programme in the Volta river basin area. *Bulletin of the World Health Organization* **64**, 667–681.

- Samé Ekobo, A., Samé Voisin, C., Eben-Moussi, E. and Ongmagne, M. J.** (1981). A propos d'un cas de méningo-encéphalite filarienne à *Loa loa*. Rappels des critères de diagnostic de certitude. *Afrique Médicale* **20**, 359–361.
- Twum-Danso, N. A.** (2003). Serious adverse events following treatment with ivermectin for onchocerciasis control: a review of reported cases. *Filaria Journal* **2** (Suppl. 1), S3.
- Wahl, G., Enyong, P., Ngosso, A., Schibel, J. M., Moyou, R., Tubbsing, H., Ekale, D. and Renz, A.** (1998). *Onchocerca ochengi*: epidemiological evidence of cross-protection against *Onchocerca volvulus* in man. *Parasitology* **116**, 349–370.
- Wanji, S., Tendongfor, N., Esum, M., Ndindeng, S. and Enyong, P.** (2003). Epidemiology of concomitant infections due to *Loa loa*, *Mansonella perstans*, and *Onchocerca volvulus* in rain forest villages of Cameroon. *Medical Microbiology and Immunology* (Berl) **192**, 15–21.
- Winkler, S., Willheim, M., Baier, K., Aichelburg, A., Kremsner, P. G. and Graninger, W.** (1999). Increased frequency of Th2-type cytokine-producing T cells in microfilaremic loiasis. *American Journal of Tropical Medicine and Hygiene* **60**, 680–686.