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# Evidence for the exacerbation of lymphedema of geochemical origin, podoconiosis, by onchocerciasis

Fidelis Cho-Ngwa\*, Alfred Ngwa Amambua, Melvin Anyasi Ambele, Vincent P.K. Titanji

Biotechnology Unit, Department of Biochemistry and Microbiology, Faculty of Science, University of Buea, P.O. Box 63, Buea, Cameroon

Received 20 April 2009; received in revised form 19 September 2009; accepted 25 September 2009

## KEYWORDS

Onchocerciasis;  
Podoconiosis;  
Lymphedema;  
NW Cameroon

**Summary** The study was conducted to investigate a variation in the distribution of endemic elephantiasis previously determined to be of geochemical origin in three neighbouring and essentially homogenous villages, Bambili, Bambui and Finge of the Bambui Health District of NW Cameroon. A total of 301 subjects were examined for onchocerciasis and lymphatic filariasis in the area using standard procedures. The onchocercal microfilarial prevalence varied from 6.5% in Bambili through 20.4% in Bambui to 60.4% in Finge. The onchocercal serological prevalence based on IgG4 detection followed a similar trend. By contrast, blood microfilariae were absent in the area as verified by use of sensitive techniques. The community prevalence of elephantiasis varied from 1.1% in Bambili to 4.4% in Bambui and 10.4% in Finge. The correlation between the parasitological prevalence of onchocerciasis and the prevalence of lymphedema in the three villages was strong ( $r=0.99$ ,  $p<0.05$ ). We confirm that the elephantiasis in the area is of geochemical origin and the results suggest that it is being exacerbated by onchocercal lymphadenitis.

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## Introduction

A recent estimate by the WHO [1] puts the number of people infected with *Onchocerca volvulus* at over 37 million. This represents a significant increase

(relative to previous estimates) in the number of people suffering from onchocerciasis, and indicates that the disease remains a public health problem in the tropics. Onchocerciasis presents a spectrum of debilitating pathologies that involve mainly the skin and eyes, but also the lymphatics [2]. These pathologies are thought to be due essentially to inflammatory reactions generated by microfilariae [2] and associated *Wolbachia* endobacteria [3].

\* Corresponding author. Tel.: +237 7762 3220;  
fax: +237 3332 22 72.

E-mail address: [chongwa\\_ub@yahoo.co.uk](mailto:chongwa_ub@yahoo.co.uk) (F. Cho-Ngwa).

The severity of onchocercal lesions follows closely the prevalence and intensity of the infection [2]. Microfilariae have been shown to invade and cause inflammations of regional lymph glands resulting in lymphadenitis in patients. It has been thought since the 1970s [4] that these inflammatory reactions may lead to elephantiasis of the extremities. However, direct evidence in support of this has been lacking.

In 1981, Price and Henderson [5] reported the prevalence of endemic, non-bancroftian elephantiasis of the legs in the Bambui Health District of North Western Cameroon. They associated the disease to the intake of toxic siliceous microparticles which leads to silicosis of peripheral lymphatics of the legs. The condition termed podocniosis and promoted by walking barefooted has been reported in other areas of red clay soils around volcanoes in Eastern Africa [6–9]. In onchocerciasis endemic areas where this soil type is not found and which are not also co-endemic for lymphatic filariasis, e.g. in some villages of the Vina, Meme and Sanaga river basins of Cameroon, endemic lymphedema and elephantiasis have not been described. A detailed description of elephantiasis in the Bambui Health District has already appeared in the literature [5,10]. The area is comprised of three villages, Bambui, Bambili and Finge. The Bambui and Bambili people have the same ethnicity and speak the Bambui–Bambili language [16]. The Finge people migrated from elsewhere. However, all three populations are socially homogenous. All three villages also have the red alluvial soil type and are located on the flanks of the same volcano. But traditionally elephantiasis in the area is known to be largely a disease of the Bambui and Finge people. To seek an explanation to the variation in the distribution of geochemical lymphedema in these essentially homogenous villages, a cross-sectional survey of onchocerciasis and lymphatic filariasis was conducted.

## Methods

### Study area and population

Before the commencement of the cross-sectional survey, ethical clearance and authorisation were obtained from the North West Regional Delegate of Health. Informed consent was obtained from each traditional authority. The purpose, nature, risks and benefits of the study were then explained to the villagers. Informed consent was subsequently also obtained from each participating adult, and

from the parent or guardian of each minor. The three neighbouring villages of the District surveyed were Bambui, Bambili and Finge. Their combined population is less than 20 000. The Bambui Health District is located some 15 km northeast of Bamenda town and is situated somewhere between longitudes 10°14' and 10°15'E and latitudes 5°6' and 6°2'N. The vegetation is forested savannah. The average altitude is about 1400 m. All three villages are located on the western slope of an elongated volcano called Sabga, which is characterized by many rapids and fast flowing streams. The soil is red and alluvial in nature for all three villages. The populations are essentially ethnically homogenous. The main occupation of all the villagers was farming, and to a lesser extent, petite trading. The study was carried out between June 2002 and September 2003.

### Clinical examination

A mobile clinic was set up at the residence of the head of each village. Eligible subjects were those born in, or had resided in the villages for at least 10 years. All eligible individuals who wilfully presented themselves to the clinic during the day were carefully and thoroughly examined for signs and symptoms of onchocerciasis and lymphatic filariasis by an expert. A structured questionnaire was completed. Free treatment for intestinal worms were included as part of the study.

### Parasitological examination and sera

The standard skin snip test for onchocerciasis was performed during the day for all eligible subjects as previously described [11]. Blood collection from the same subjects for blood microfilaria identification took place at night between 10:00 pm and 2:00 am. Thick films of capillary blood were prepared, stained with Giemsa and examined by microscopy. Five (5) ml of peripheral blood were further collected from each subject by venepuncture. The Knott's concentration technique was performed with 1 ml of the blood following standard procedures. The remaining 4 ml of blood were processed for sera as described previously [11]. Normal African Sera for determination of background reactivity in ELISA were prepared similarly from the blood of 14 subjects from Molyko-Buea, Cameroon, a non-transmission urban area. These controls were negative for onchocerciasis by the skin snip test, and had previously been shown to be serologically negative by the OvH2-based test of Nde et al. [12].

**Table 1** Demographic profile of study subjects.

Community	Population estimate	Number examined	Geometric mean of age (in years)	Age range (years)	Males/females
Finge	3000	96	38.9	13–72	32/64
Bambui	8000	113	36.6	11–75	54/59
Bambili	8000	92	36.4	10–70	42/50

**Table 2** Clinical profile of study subjects.

Community	Number with dermatitis	Number with eye disease	Number blind	Number with lymphadenitis	Number with hydrocele	Number with lymphedema
Finge	87 (90.6%)	71 (74.0%)	02 (2.1%)	33 (34.4%)	3	10 (10.4%)
Bambui	54 (47.6%)	50 (42.7%)	01 (0.97%)	16 (14.2%)	2	05 (4.4%)
Bambili	71 (77.2%)	40 (43.5%)	06 (6.5%)	4 (4.3%)	0	01 (1.1%)

### *O. ochengi* total extract

*O. ochengi* crude extract was used in detection of *Onchocerca* specific IgG4 antibodies by ELISA. It was prepared essentially as described previously [13]. Briefly, isolated adult worms were frozen under liquid nitrogen and then crushed to fine powder on dry ice using a plastic mortar and pestle. Two buffers – Tris–saline buffer (25 mM Tris–Cl, 125 mM NaCl, pH 7.4) for fraction F1 and Tris–saline–SDS buffer (25 mM Tris–Cl, 125 mM NaCl, pH 7.4, containing 0.5% [w/v] SDS) for fraction F2 – were used to sequentially homogenize the worm material on ice. Crude extracts were obtained for each buffer after centrifugation at 6000 × g. Equal volumes of fractions F1 and F2 were mixed to obtain *O. ochengi* total extract (OOTE).

### Enzyme linked immuno-sorbent assay (ELISA)

ELISA was carried out essentially as described by Cho-Ngwa et al. [14] with slight modifications as follows: plates were coated with OOTE immediately after it was prepared and the enzyme substrate was para-nitrophenyl phosphate (PNPP) (SIGMA, Germany) which was used according to the

manufacturer's instructions. Optical densities were read at 405 nm (OD<sub>405</sub>). The results were analysed statistically using the Microsoft™ Excel software (Microsoft Corporation, USA).

## Results

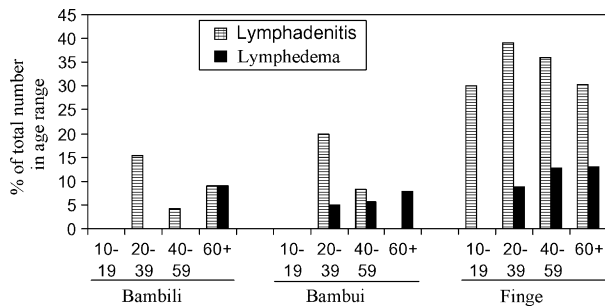
### Clinical and demographic profiles of study subjects

Of the total of 301 subjects involved, 96 were examined in Finge, 113 in Bambui and 92 in Bambili. Table 1 summarises the demographic profiles of the subjects. The clinical examinations revealed that *Onchocerca* nodules were rare in the area while dermatitis was common. Eye disease, including cases of conjunctivitis was also common (Table 2). Lymphedema at different stages were observed as previously described [5,10]. Only one patient in Finge could be classified as having elephantiasis and this was characterized by pronounced swelling of the legs, with disfigurement, thickening and hardening/fissuring of the skin of both legs. The incidences of lymphadenitis in all three villages peaked in the age range of 20–39 (Fig. 1), while for

**Table 3** Parasitological profile of study subjects.

Community	Number with skin mf <sup>a</sup>	Mean skin mf/snip for community	Range, skin mf/snip	Number with blood mf	Number with nodules	Mean nodules/subject
Finge	58 (60.4%)	6.83	0–100	0	7	1.9
Bambui	23 (20.4%)	5.54	0–200	0	3	1
Bambili	6.0 (6.5%)	0.14	0–7	0	0	0

<sup>a</sup> Prevalence in brackets; mf: microfilaria.



**Figure 1** Age stratified prevalence of lymphatic disease in the three villages.

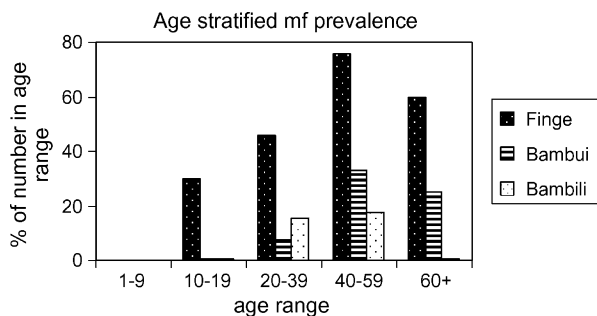
lymphedema, the incidences generally increased with age to peak at old age (60+) (Fig. 1).

**Parasitological profiles**

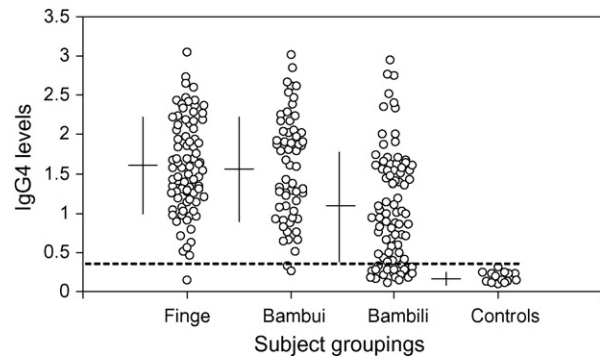
The onchocercal microfilarial prevalence varied from 60.4% in Finge, 20.4% in Bambui to 6.5% in Bambili. By contrast, no blood microfilaria was found in all the 301 subjects. Individual skin microfilarial loads for positive cases ranged from 0 to 200 mf/snip in the entire study area (Table 3). The community microfilarial loads followed the trend in community microfilarial prevalences. For all three villages, mf prevalences rose to peak at age range 40–59 then fell (Fig. 2). Microfilarial loads also rose to age range 40–59 then fell. The most important vector-breeding site was a large fast flowing stream at the foot of Finge.

**Serological prevalences for onchocerciasis**

As expected, the village serological prevalences of onchocerciasis based on IgG4 detection were much higher than the parasitological (Fig. 3). However, both sets of prevalences were well correlated ( $r=0.77$ ,  $p<0.05$ ). The serological prevalences varied from 98.8% in Finge, 96.6% in Bambui to 78.6% in Bambili. The assay sensitivities amongst the microfilarial positive cases were 100% for all three



**Figure 2** Age stratified microfilarial (mf) prevalence given as % of total number in age range.

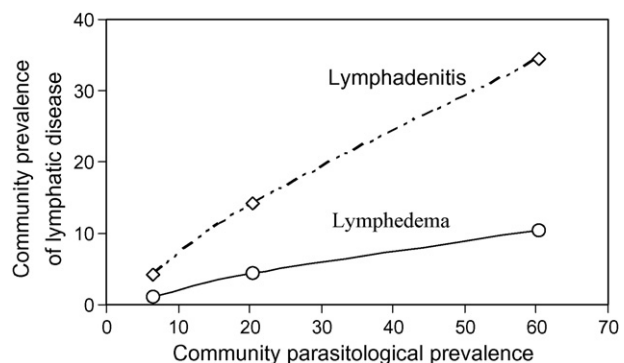


**Figure 3** Individual *Onchocerca*-specific IgG4 levels in the three communities. Long dotted horizontal line indicates the mean+2 SD of the normal African controls employed as cut-off point in the assays. Short solid horizontal lines indicate group means, with SD as vertical solid lines. SD = standard deviation.

villages. Thus, the IgG4-based assay was capable of detecting an additional 38.4% of putative onchocerciasis patients in Finge, 76.2% in Bambui and 72.1% in Bambili.

**Correlations between lymphatic diseases and onchocerciasis prevalence**

There were strong correlations between the onchocerciasis parasitological prevalences of the villages and the prevalences of both lymphedema and lymphadenitis. The respective correlation coefficients were 0.994 and 0.997 for lymphedema and lymphadenitis respectively (Fig. 4). These correlations suggested strongly that lymphedema in these populations could be exacerbated by onchocerciasis or onchocercal lymphadenitis.



**Figure 4** Strong positive correlations between the community parasitological prevalences of onchocerciasis and the community prevalences of lymphedema ( $r=0.99$ ;  $p=0.035$ ), and lymphadenitis ( $r=0.997$ ;  $p=0.024$ ).

## Discussion

In the present paper we describe an association between the endemicity of onchocerciasis and of elephantiasis of geochemical origin (podoconiosis) in three neighbouring villages making up the Bambui Health District of North Western Cameroon where lymphedema (and elephantiasis) that was previously determined to be podoconiosis is endemic. The results obtained confirmed the absence of *W. bancrofti* in the area and fortifies previous claims that the lymphedema is of geochemical origin. This strong correlation ( $r=0.994$ ,  $p<0.05$ ) suggested an exacerbating role for onchocercal lymphadenitis on podoconiosis.

In 1981, Price and Henderson reported the prevalence of endemic elephantiasis of geochemical origin known as podoconiosis in this District. They refuted previous claims that the elephantiasis in the area was due to onchocerciasis. The classification of the elephantiasis in this Health District as podoconiosis has been based on a combination of observations or facts: Firstly, the condition is endemic in other areas of red clay soils around volcanoes in Africa [6]. This type of soil was observed in this District. In East Africa, podoconiosis was not linked to the filariasis. Thus it can exist alone, provided the risk factors are present and irrespective of whether there is filariasis. Secondly, in other areas in Cameroon where onchocerciasis is hyperendemic, but which do not have this soil type, e.g. in villages of the Meme, Vina and Sanaga river basins, there are no reports of endemic lymphedema or elephantiasis. Only lymphadenitis and hanging groins are observed to be endemic. Thus the condition seems to be tied more to the nature of the soil/barefoot walking than to the endemicity of onchocerciasis. In 2000, Ivoke [15] published a contradictory paper reporting an overall *W. bancrofti* prevalence of 14.5% in the same Health District. Interestingly, Ivoke [15] did not report on the hypo- to hyperendemic level of onchocerciasis in the area. The studies of Price and Henderson [5] and ours were conducted before the commencement of mass ivermectin treatment in the area. It is thus possible that Ivoke [15] erroneously classified *O. volvulus* microfilariae as those of *W. bancrofti*. Previous studies have suggested that onchocerciasis can lead to, or predispose a patient to elephantiasis [4]. The mechanism by which this can result is unclear, but it is suggested that the inflammatory reactions that lead to lymphadenitis in onchocerciasis may facilitate the deposition of siliceous material in the lymphat-

ics thereby promoting the obstruction of the latter and leading to elephantiasis of the lower limbs. A more recent parasitological survey subsequent to the present in the same area and neighbouring villages has also confirmed this absence of *W. bancrofti* microfilariae [10].

Our data associate the variation in the community prevalences of podoconiosis and lymphadenitis to the variations in both the serological and parasitological prevalences of onchocerciasis (Fig. 4). The latter is hyperendemic in Finge (60.4% parasitological prevalence), mesoendemic in Bambui (20.4%) and hypoendemic in Bambili (6.5%). This trend was expected since the main vector-breeding sites were closer to Finge and since the natives of Bambui and Bambili live closer to each other. In the District, lymphedema is known to be largely a disease of the Bambui and Finge people. As these three villages are socially homogenous, with the same geochemical factors, and with the Bambui and Bambili people being of precisely the same ethnicity, one would have expected the prevalence of any lymphedema of geochemical origin to be uniform or follow some genetic trend. This was however not the case, and the strong correlation observed could reasonably be interpreted as onchocerciasis being at least a contributory factor, especially as it has been suggested in the past to be a possible causative factor. Unfortunately, these were the villages of homogenous geochemistry that could reasonably be included in the study, such that the number of study points remained few.

Overall, the IgG4-based test detected by far more positive cases than the skin snip test, with the difference being more pronounced in the hypo- and mesoendemic villages. And while confirming a relatively higher number of IgG4 positive individuals in the hyperendemic than mesoendemic areas, this test appear not to be able to resolve between hyperendemicity and mesoendemicity of onchocerciasis. The large difference between the parasitological and serological tests confirms the difficulty in using IgG4-based tests in distinguishing between active infections and exposure.

It can therefore be concluded that bancroftian filariasis is not endemic in the Bambui Health District of NW Cameroon, and that the endemic elephantiasis in the area confirmed to be of geochemical origin is apparently being exacerbated by onchocerciasis. Consequently, the very low level of elephantiasis in Bambili would suggest that the effective control of onchocerciasis in the area could result in a more or less parallel control of the endemic lymphedema.

## Conflict of interest

**Funding:** This study was realised thanks to a grant from the International Science Programme (ISP) of Uppsala University, Sweden (Project CAM01).

**Competing interests:** None declared.

**Ethical approval:** Ethical approval was given by the North West Regional Delegate for Health to Professor Vincent P.K. Titanji.

## Acknowledgement

We are grateful to the people of the Bambui Health District for their collaboration in the study.

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