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Does onchocerciasis transmission take place in hypoendemic areas? A study in North Region of Cameroon

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Abstract

OBJECTIVE: Community-directed treatment with ivermectin (CDTI) for onchocerciasis control is targeted to meso and hyperendemic areas in Africa. Below the threshold, communities are considered hypoendemic and mass treatment is not recommended. As policy begins to shift from control to elimination, hypoendemic areas’ role in maintaining *Onchocerca volvulus* needs re-examination. The study determined whether independent transmission occurs in a hypoendemic area in the north region of Cameroon.

METHODS: Ten “high risk” communities along the River Mayo Douka system in Ngong Health District, at least 20 km from the nearest CDTI program that has been implemented for over 15 years were studied. 649 adults (over 20 years of age) and 561 children (under 10

years) resident of the communities were examined for nodules and microfilaria. A subsample of 334 adults was examined for onchocercal ocular morbidity. *Simulium* flies from four collection points were captured over three months yearly for two years, and examined by dissection for larval stages of *O. volvulus*.

RESULTS: Nodule and microfilariae (mf) prevalence among adults was 12.20% and 2.91%, and children 9.2% and 0.48% respectively. Blindness due to onchocerciasis was insignificant, although low rates of chronic onchocercal ocular disease (<2%) were observed. Four (0.16 percent) out of 255 flies collected in 2008 were infected with L3 larval stage, and one black fly out of 39 collected in 2009 had two L2 larval stage morphologically consistent with *O. volvulus*.

CONCLUSION: Ngong is a 'hypoendemic' focus with likely low grade indigenous transmission in isolation from meso/hyperendemic areas. Consequently transmission from hypoendemic areas could contribute to rapid disease recrudescence in the post treatment phase of adjacent former meso and hyperendemic areas.

Introduction

Onchocerciasis, also known as river blindness was one of the world's second leading infectious cause of blindness until a very successful international campaign was launched against it decades ago. The infection is caused by a nematode worm known as *Onchocerca volvulus*, which lives up to fifteen years in the human host. The infective larval stages of *O. volvulus* are transmitted to humans through the bite of certain species of female *Simulium* flies that breed in fast running rivers and streams (Hopkins et al 2007). These infective larval stages develop into adult male and female worms that group in subcutaneous clusters known as 'onchocercomas' or 'nodules' that are often, but not always palpable. Females release microfilariae (mf) that migrate from the nodules into the skin, eyes, and lymph nodes where they may produce severe and progressive inflammatory lesions. Acute papular dermatitis and severe pruritus are often the first manifestations as a result of a strong immune response to dead mf, often leading to spotty depigmentation (leopard skin) or dermal thickening and wrinkling with scaling referred to as "lizard" skin (Brieger, et al 1998). Mf can also enter the eyes causing ophthalmologic complications that can involve both the anterior and posterior segments of the eye (Boatin and Richards, 2006).

Ivermectin (Mectizan®) donated by Merck & Co. is currently the drug of choice for treating onchocerciasis (Thylefors et al 2008). For the best public health effect, the World Health Organization recommends mass treatment of all eligible persons (e.g. persons not seriously ill, children over five years of age, non pregnant women, and lactating mothers a week after delivery) of entire communities. The main strategy in countries assisted by the African Programme for Onchocerciasis Control (APOC) is distribution of a single annual dose of

ivermectin through community-directed treatment with ivermectin (CDTI) in areas where onchocerciasis is considered to be a public health problem: these areas are deemed 'meso/hyperendemic' and have a nodule rate $\geq 20\%$ and/or a microfilaria prevalence of $\geq 40\%$. In those areas, the populations are at greatest risk of developing ocular or skin manifestations, so that ivermectin treatment is a priority. In hypoendemic areas (below meso/hyperendemicity threshold), the risk of morbidity is to a large extent reduced.

Targeting for mass treatment through CDTI was through country by country, large scale Rapid Epidemiological Mapping of Onchocerciasis (REMO) to detect these meso/hyperendemic areas (Ngoumou et al, 1994). REMO was rooted in the fact that the vectors for onchocerciasis have highly specific breeding site requirements and limited flight range, and therefore it was possible with the aid of topographical maps to choose representative communities most likely to be seriously affected by onchocerciasis. The REMO was followed by the Rapid Epidemiological Assessment (REA) that relied on palpation examinations for characteristic onchocercomas ("nodules") in a sample of 30-50 males aged 20 years and over in the 'high risk' or 'first line' communities (located on the rivers) drawn from areas likely to have the highest transmission and thus greatest risk for transmission and severe disease (Boatin and Richards, 2006).

What has remained unstudied is the uncertain transmission in hypoendemic areas (nodule rate $< 20\%$ and microfilaria prevalence of $< 40\%$) and the presumed low onchocerciasis morbidity that led to their exclusion from mass treatment. Richards et al., 2000 assumed that transmission was likely to be ongoing in some of these areas. As policy begins to shift from control of morbidity to complete transmission interruption, the role that hypoendemic areas excluded from the APOC program play in independently sustaining *O. volvulus* needs to be carefully examined. If independent transmission existed in hypoendemic areas, onchocerciasis could be 'reseeded' into adjacent meso/hyperendemic areas (that presumably have higher vector biting

rates) should the decision be made to halt mass treatment with ivermectin. The aim of the present study was to determine whether independent transmission occurs in a hypoendemic area not targeted for mass treatment by ivermectin in northern Cameroon.

Methods and Materials

Study area: The study took place in North Region of Cameroon in the Ngong Health District, which has a population of about 20,000 people in about 300 km² (Figure 1 and 2). “No CDTI” areas shown in Figure 1 may or may not contain hypoendemic areas. Ngong hypoendemic focus was selected based on a review of REMO and REA data conducted in the 1990s which showed that onchocerciasis nodule rates in the area occurred in apparently hypoendemic fashion (<20% nodule rate), and nearby meso/hyperendemic areas targeted for mass treatment were at least 20 km away from Ngong focus (Ngoumou et al 1994; Macé et al 1997, APOC's REMO website- <http://www.who.int/apoc/cdti/remo/en/index.html>). Those meso/hyperendemic areas have been under mass treatment with ivermectin for at least 15 years, with treatments first launched with support from the River Blindness Foundation and subsequently expanded with The Carter Center and APOC assistance.

Study sample: The methods recommended for rapid epidemiological mapping (REMO), and rapid epidemiological assessments (REA) were applied (WHO Report, 1991; WHO Report, 1992; Ngoumou et al, 1994; Abanobi, 1999). There were less than twenty communities in Ngong hypo endemic focus, and ten of them along R. Mayo Douka and its distributaries were selected for the study. These were well-established communities with no evidence of significant population mobility that have never been under CDTI. Beyond 10 km on both sides of R. Mayo Douka are uninhabited farmlands and savanna woodlands. The selected communities were “first line” communities from R. Mayo Douka, and supposedly “high risk” for onchocerciasis. Health education about onchocerciasis was given and the purpose of the study explained to local leaders and community members in each community in a general

meeting. After consent was obtained, 42 to 120 adults per community, 20 years of age and above, who had lived in them respectively for at least 10 years were examined. There were no refusals among adults. About 80% of the adults in every selected community were examined. In addition, children (3 to 10 years old) whose parents had assented to their participation were enrolled in the study. A few children who did not participate did not affect the results of the study. In total, 1210 persons (649 adults and 561 children) were examined for onchocercomas by palpation and mf by skin snip. Ocular morbidity related to onchocerciasis was assessed in a subsample of 334 adults in six of the communities, and *Simulium* flies were collected by human landing capture for dissection in sites located in four of the ten communities.

Nodule palpation: Nodule palpation was performed in an enclosed and private area to search for nodules of onchocerciasis by physical (palpation and inspection) examination. Nodules were ‘suspected’ onchocercomas (not confirmed by dissection or biopsy) defined as being firm, often flattened or bean-shaped, usually movable, non tender and up to several centimeters in diameter. They were distant from usual locations of lymph nodes (neck, axillae, inguinal) by being palpated on bony prominences of the ribs, iliac crests, sacrum and upper leg (Albiez et al. 1988).

Skin snipping: After cleansing the skin with alcohol, the tip of a sterile lancet needle mounted in a holder was used to elevate 3-4 mm of skin over the right posterior superior iliac crest. A sterile surgical razor blade was then used to remove a skin snip at the base of the elevation. The skin, dangling from the tip of the needle, was transferred to a well of 96 microtiter plate containing sterile normal saline solution. The blade and needle were then used to obtain the second specimen on the left side in the same manner, after which the needle and blade were discarded in an appropriately safe ‘sharps’ container (Schulz-Key 1978 and WHO 1995). The skin snips were kept at room temperature in the microtiter plate in normal saline solution for 12

– 24 hours in order to allow any mf present to emerge from the skin. Each skin snip was then removed from the well with a needle, and the saline solution was examined unstained under a microscope (40x) for mf of *O. volvulus*. The results were expressed as positive/negative, and as a proportion of the number of persons in the sample.

Ocular morbidity assessment: Although ocular morbidity is not an indicator of transmission, we were compelled to evaluate the ocular status of residents in Ngong since North Region is known to have a blinding strain of *O. volvulus* (Chippaux et al, 1999). The objective was to confirm that indeed morbidity (as a late stage of infection) did not occur in a hypoendemic region that has never been treated with ivermectin. 334 adult male participants from 6 communities agreed to slit lamp examinations. All examinations were preceded by health education and consent, after which each participant sat with his head lowered between the knees for 5 minutes to facilitate the descent of mf in the anterior chamber to a point where their observation was possible. The participants then were examined by an experienced ophthalmologist using a slit lamp under 25x magnification. The eyes were not dilated and the focus of the examination was on the anterior segment of the eye. Results recorded include mf in the anterior chamber, mf in the cornea (with or without inflammation), iritis, and sclerosing keratitis. Corneal stromal opacities (punctuate keratitis) in which mf fragments were not visible were not counted as onchocerciasis-related (Winthrop et al 2006). Patients with conditions that required immediate treatment were referred to the nearest hospital.

Entomological Assessment: After a review of local drainage systems into the Mayo Douka river using a 1:200,000 map, followed by field reconnaissance along the main river, the study team selected four *Simulium* fly collection sites, located in four out of ten "firstline" communities (Mayo Ouro Malloum, Mayo Ouro Be, Mayo Douka, and Mayo Ouro Bororo).

Selection of the *Simulium* fly collection sites was based on proximity to the community, favourable river flow, and other ecological conditions necessary for black fly breeding. Four teams of two fly collectors (one team per community) were recruited, consented, trained, and equipped to perform landing captures of *Simulium* flies. The collectors were at least 18 years of age, and informed that they could opt out of the study if they so wished at any time, without any repercussions. The collectors sat at the 4 selected sites near the river bank and exposed their legs between 0800 – 1200 and 1400 – 1800 hours, three days every two weeks per month from late August to mid-November, 2008 and July to November, 2009 (WHO Report, 1995). Female *Simulium* flies seeking blood for their eggs would come and settle on the exposed human legs. Before being bitten, the trained collectors would use suction tubes to catch the *Simulium* flies, and then preserve them in isopropanol (in 2008), or ethanol (in 2009) in batches of up to 50, as immediate dissection was not possible. The tubes in which the flies were stored were labeled by catching site, date and time. In the laboratory, the flies were stained with Mayer's hematoxylin, dissected and examined for onchocercal larval stages in the head, thorax and abdomen (Davies, 1995). Mayer's hematoxylin ensures progressive nuclei stain and improves visibility of all forms of *O. volvulus* in the *Simulium* fly stored in alcohol. The reading of flies collected in 2008 preserved in isopropanol allowed only observation of L3 larvae. In 2009, we used ethanol preservative which allowed us to observe all the larval stages with Mayer's hematoxylin.

Ethical Approval: The study was approved by the Emory University Institutional Review Board (eIRB - 11438) and the Ministry of Health of the Government of Cameroon, Younde.

Results

Microfilariae (mf) and nodule prevalence: The mean mf prevalence among adults was 2.91%, ranging from 0 to 11.8% in ten communities (Table 1). None of the communities met the 40% mf prevalence criteria for mesoendemicity (and the threshold for CDTI). The mean nodule prevalence in the same group was 12.2% (range 5.3% to 27.1%). In contrast to mf results, two of the ten communities had a nodule prevalence of over 20%, which exceeded the 20% threshold that is the currently accepted indicator for CDTI (and the threshold for mesoendemicity). Only three of 516 children (0.47%) under ten years of age had mf detected in their skin snips (community range of 0 to 1.9%). Nodule rates in children, as with adults, were more than anticipated with a mean of 9.2% (range from 1.6% to 17.5%). Four communities exceeded 10% nodule prevalence among children (Table 2).

Ocular Morbidity: Three hundred and thirty four persons were examined, with an average of 55 persons examined per community (range 34 to 66). One person (0.3%) had mf in the anterior chamber and another (0.3%) mf in the cornea. Among chronic ocular disease findings specific to onchocerciasis, the results showed that 4 (1.2%) had iritis, and 2 (0.6%) sclerosing keratitis (Table 3).

Entomological assessment: 255 and 39 *Simulium damnosum s.l.* flies were caught during 2008 (late August to mid-November) and 2009 (July to November) respectively and preserved for staining and dissection (Figure 3). Four infective (L3) larvae were found in four flies (1.57% infective rate) in 2008. In 2009, one *Simulium damnosum s.l.* fly had two L2 larvae in the thorax; no L3 were found.

DISCUSSION

Hypoendemicity and transmission

The results show that REMO studies conducted under APOC guidelines in northern Cameroon properly classified the ten communities we surveyed in Ngong Health District as hypoendemic (mf prevalence <40%) for onchocerciasis, although 2 of the communities had nodule rates greater than 20%. Based on the REMO maps, Ngong has not been included in the CDTI program. However, the study also demonstrated disquieting findings that suggest that low level transmission of *O. volvulus* is ongoing in Ngong as based on the findings of mf in skin snips from three children younger than ten years of age, as well as L2 and L3 larval stages observed in locally caught *Simulium damnosum s.l* vectors. Although ocular morbidity was insignificant, both acute (mf in the anterior segment of the eye) and chronic (iritis and sclerosing keratitis) ocular disease specific to onchocerciasis were observed among Ngong residents.

We favor the hypothesis that the source for these human and vector infections is active low grade transmission within the Ngong health district itself. Alternatively, since *S. damnosum* is known to fly long distances in this part of Africa, the vectors could have been infected over 20 km away in the nearest meso/hyperendemic zone. That assumption would assume active transmission in areas that have been under annual distribution of ivermectin for at least 15 years with yearly treatment coverage reported to be >90% of eligible population. Therefore, it is reasonable to believe that onchocerciasis in Ngong is from local autochthonous transmission originating from infected people and flies circulating in and around the untreated communities. Therefore, if a new goal of APOC will be elimination of onchocerciasis transmission, the

nature of the REMO map of Africa needs to be reexamined. Treatment areas are not necessarily the only areas where transmission is ongoing, and new investment will be needed to redefine and expand the CDTI program to many of the areas previously left untreated.

Consideration is now being given to stopping CDTI in areas that have been treated with good coverage for over 15 years (WHO Report, 2009; Diawara et al 2009). We suggest that stopping ivermectin in formerly meso/hyperendemic areas that are adjacent to hypoendemic areas like Ngong that have low grade autochthonous transmission could result into "reseeding" of the parasite into those post treatment zones. The result could be prompt disease recrudescence. One option could be application of twice yearly treatment with ivermectin in adjacent areas of low transmission to hasten 'catching up' with the epidemiological trend in nearby and former meso/hyper-endemic areas (Cupp and Cupp, 2005).

Only 294 *Simulium* flies were collected in 8 months of intermittent field activities during 2008 and 2009. A longer period of study could provide better data on annual biting, transmission and infection rates (Renz, 1987) and we recommend future studies to assess entomological and environmental indicators throughout the year, to include activities in the meso/hyperendemic areas in the vicinity of Ngong, over a period of at least two years if possible. This could also reveal changes in rainfall period, how it may impact the development of larval stages of *Simulium damnosum s.l.*, and the ability to transmit onchocerciasis within or reseed former meso and hyperendemic areas if ivermectin treatment was halted.

Confounding factors: In the study, nodule rates were higher than expected given corresponding skin snip derived mf prevalences, especially in children. This could have been confounded by the presence of ganglia in some communities. Ganglia can form around any joint, and are usually painless and often barely visible as localized swellings. They usually do

not appear to be inflamed as can be lymph nodules (Busson, Bourée and Doyon, 1978). The study team did not dissect nodules to confirm their etiology by identifying the presence *O. volvulus* worms, and therefore future studies should be carried out to determine the actual onchocercosis prevalence rates. We also recommend that future entomological studies include molecular techniques to determine if the larvae being observed in *Simulium* dissections are indeed those of *O. volvulus*, and not of the cattle parasite *O. ochengi*.

***Loa loa* co-endemicity:** The Cameroon onchocerciasis campaign has been plagued by the presence of *Loa loa* and the fear of post treatment encephalopathy. However, in case of North Region in general and Ngong hypoendemic focus in particular, *Loa loa* does not occur (Thompson et al 2004). In onchocerciasis endemic areas in Cameroon where *Loa loa* does occur, our recommendations to treat in onchocerciasis hypoendemic areas would need reexamination. It is possible that mass treatment with doxycycline could eliminate the occurrence of post treatment encephalopathy (Wanji et al 2009). However, this is in no way underestimating the challenge that *Loa loa* co-endemicity with onchocerciasis in some foci presents should an elimination policy for onchocerciasis be adopted in Africa.

In conclusion, our findings show that autochthonous onchocerciasis transmission in hypoendemic areas may exist, and may not entirely depend on the transmission generated from nearby meso/hyperendemic areas. These hypoendemic areas need to be identified and aggressively treated, perhaps with twice per year ‘catch up’ CDTI treatment regimens. Transmission from hypoendemic areas could contribute to rapid disease recrudescence in the post-treatment phase of adjacent former meso/hyperendemic areas.

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Legend

Figure 1: Figure 1: Rapid Epidemiological Map of Onchocerciasis (REMO) of Cameroon showing: definite CDTI areas (meso and hyperendemic), no CDTI areas (hypoendemic) including Ngong study area , and excluded areas (not endemic for onchocerciasis).

Figure 2: Map of North Region of Cameroon showing the Ngong onchocerciasis hypoendemic focus.

Figure 3: Ngong onchocerciasis hypoendemic focus *S. damnosum* s.l captures and rainfall 2008-2009

 Monthly *Simulium* flies collected during 2008.

 Monthly *Simulium* flies collected during 2009.

 Rainfall (mm) during 2008

 Rainfall (mm) during 2009

Figure 1

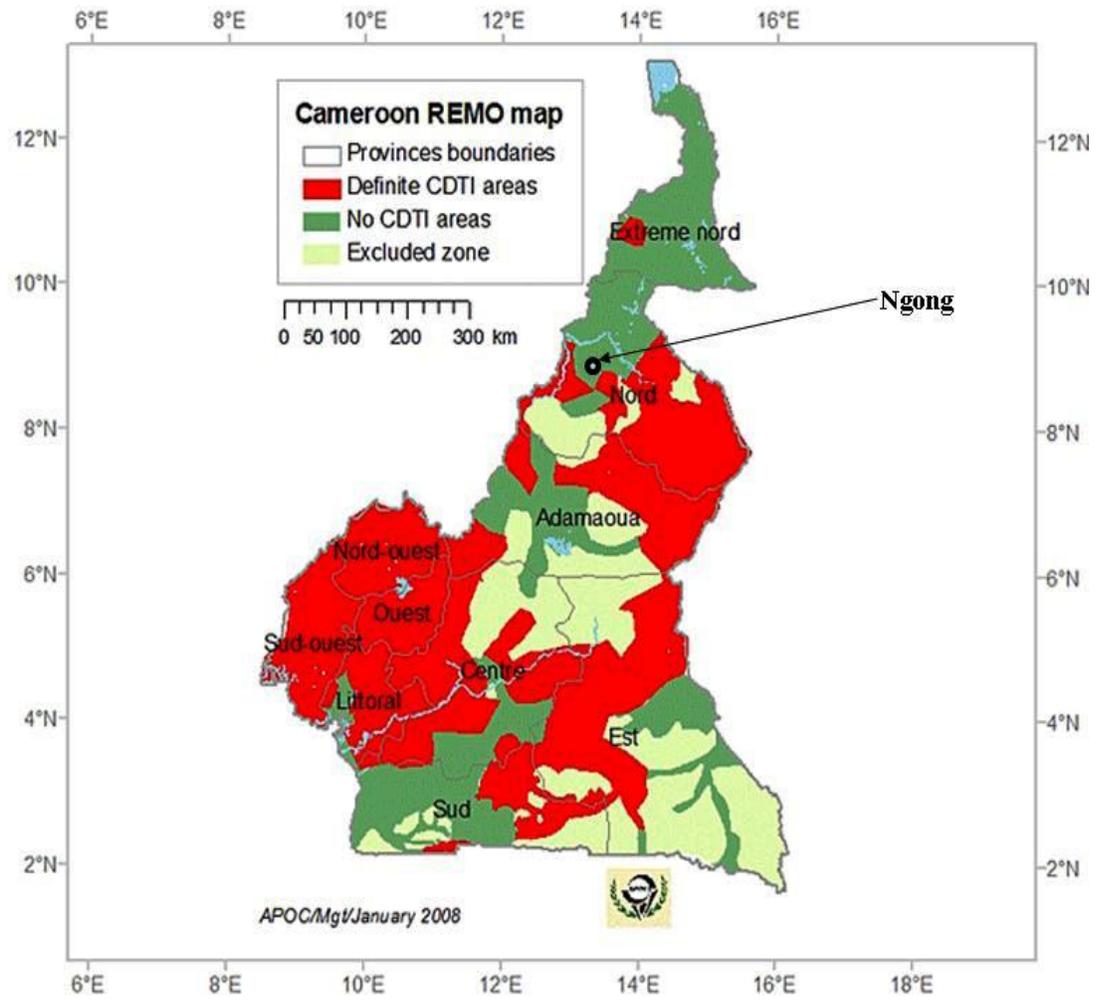


Figure 2

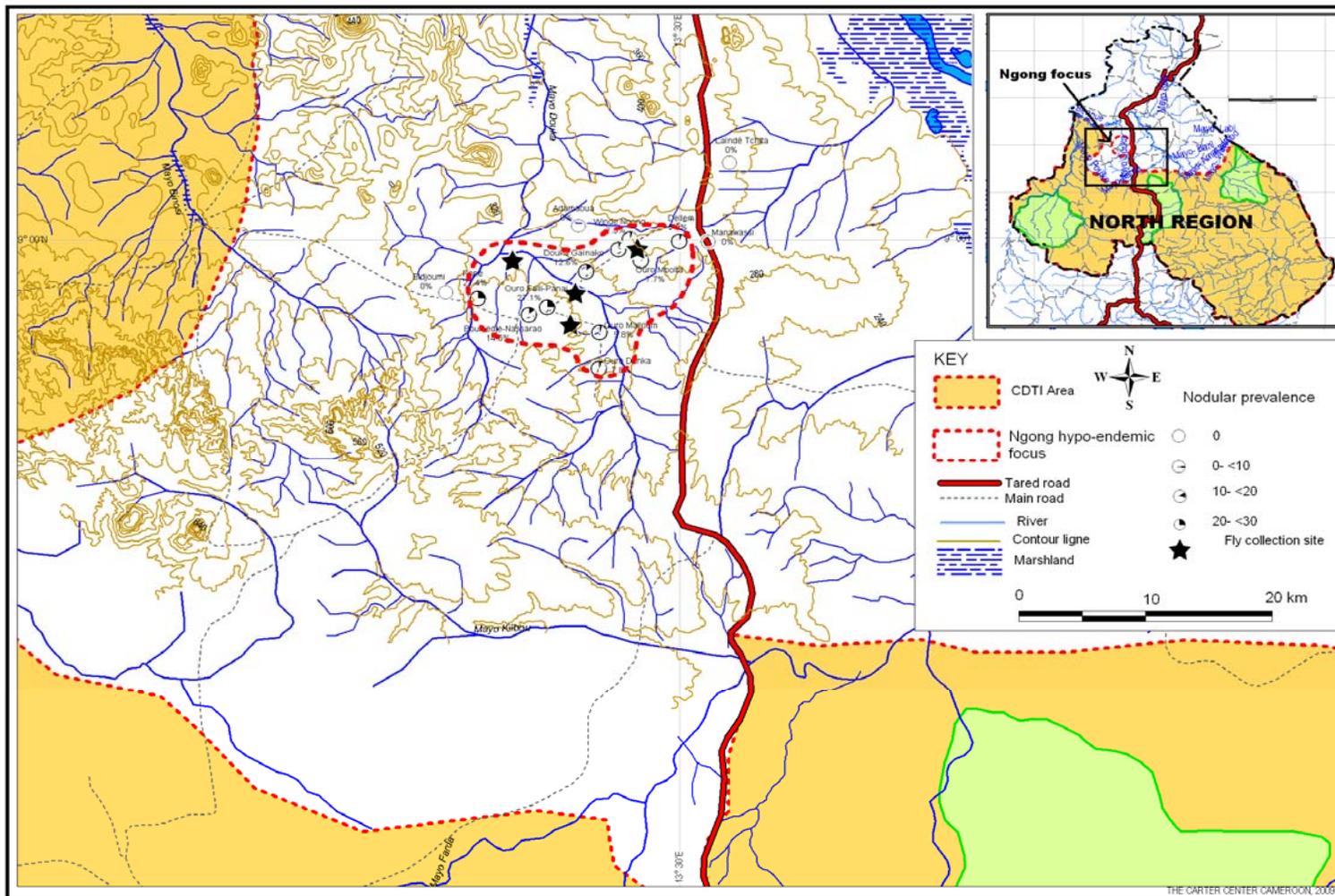


Figure 3

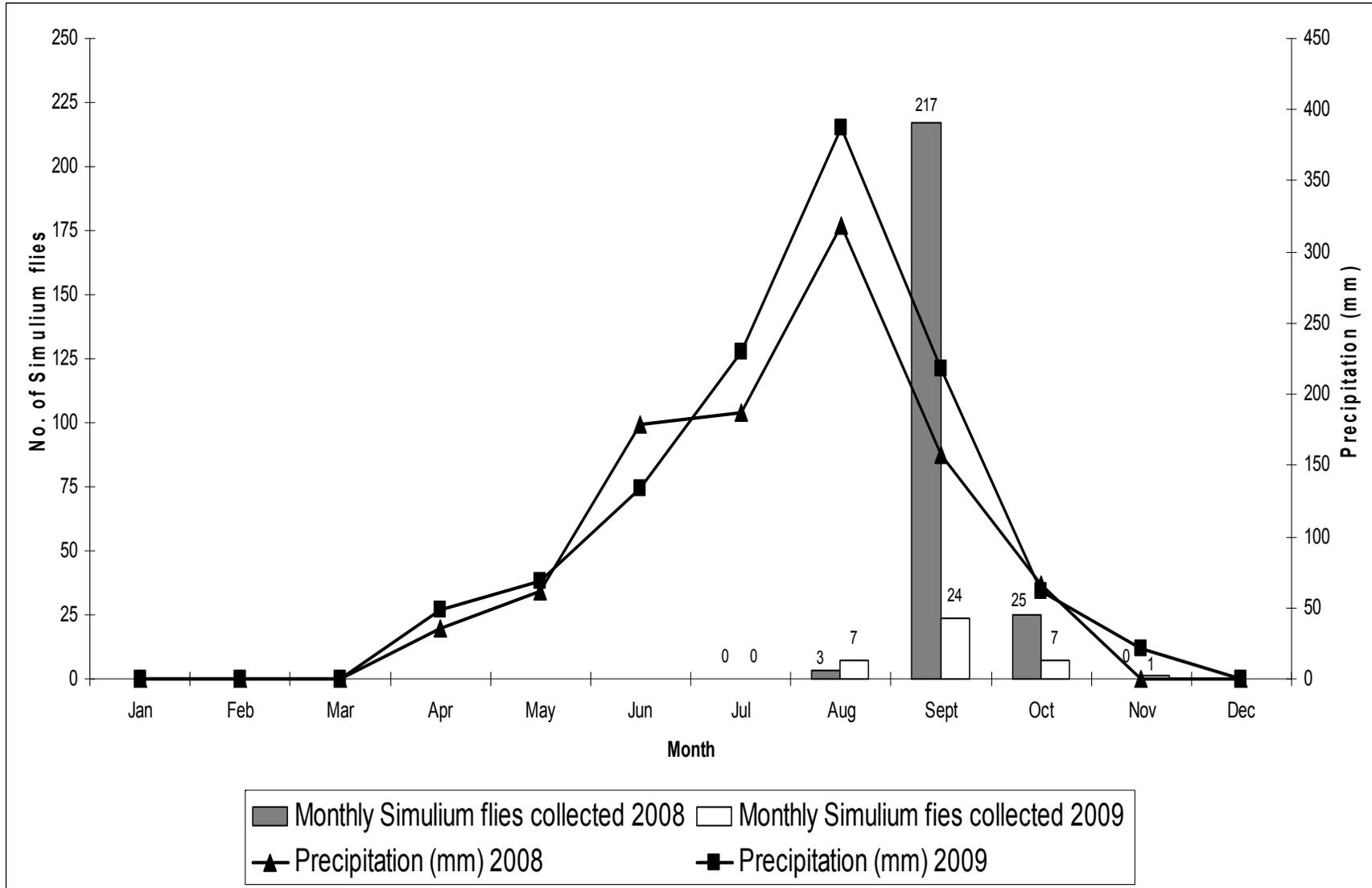


Table 1: Microfilariae and nodule prevalence in 649 adult \geq 20 years old

Community name	No. of Persons examined	Mean age	No. Positive (mf)	% mf positive	No. Positive (Nodules)	% nodule positive
Winde Ngong	55	41.54	0	0.00	4	7.30
Ouro Malloum	51	35.76	1	1.96	5	9.80
Ouro Falli-Panai	48	39.37	2	4.17	13	27.10
Ouro Donka	51	36.35	6	11.76	4	7.80
Koubadje	120	37.07	1	0.83	10	8.30
Kone	42	42.78	2	4.76	9	21.40
Douka Gaïnako	87	35.22	2	2.30	11	12.60
Dellem	75	38.48	0	0.00	4	5.30
Ouro Mbolta	65	37.46	1	1.54	5	7.70
Boumedje-Nassarao	55	38.41	1	1.82	8	14.6
	649		16	2.91	73	12.2

Table 2: Microfilariae and nodule prevalence in 561 children, 3-10 years old.

Community Name	No. of Children	Mean age	No. positive	% mf	No. positive (nodules)	% nodules
Winde Ngong	57	7.56	0	0	10	17.54
Ouro Malloum	67	7.73	1	1.49	5	7.46
Ouro Falli-Panai	52	7.42	1	1.92	4	7.69
Ouro Donka	14	5.57	0	0	1	7.14
Koubadje	79	6.91	1	1.27	5	6.33
Kone	55	6.69	0	0	3	5.45
Douka Gainako	63	7.79	0	0	11	17.46
Dellem	62	7.75	0	0	1	1.61
Ouro Mbolta	53	8.05	0	0	6	11.32
Boumedje-Nassarao	59	7.59	0	0	6	10.2
	561		3	0.47	52	9.2

Table 3: Ocular lesions specific to onchocerciasis in 334 adults of ≥ 20 years old

Community Name	No Assessed	Mean Age	No. of mf in anterior Chamber	% mf present in anterior Chamber	No of Stage A+B specific punctate keratitis	% Stage A+B specific punctate keratitis	No. of Scelerosing keratitis cases	% sclerosing keratitis
Ouro donka	34	32.9	0	0.00	0	0.00	0	0.00
Ouro Falli-Panai	61	34.1	0	0.00	0	0.00	0	0.00
Bounmedje-Nassarao	50	23	0	0.00	0	0.00	1	2.00
Ouro Malloum	66	25.5	1	1.52	0	0.00	0	0.00
Douka Gainako	62	27.4	0	0.00	0	0.00	1	1.61
Kone	61	27.8	0	0.00	1	1.64	0	0.00
Total	334	28.45	1	0.30	1	0.30	2	0.60