Loa loa and *Mansonella perstans* filariasis in the Chaillu mountains, Congo: parasitological prevalence

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Abstract

1934 Bantus and 379 Pygmies were investigated for *Loa loa* and *Mansonella perstans* filariasis in 7 villages in the Chaillu forest of the Congo. Bantus were more frequently infected with *L. loa* than Pygmies (18·9% of microfilariae carriers compared with 10·6%). In individuals over 30 years of age, males were more frequently infected than females. Microfilarial densities increased until the age of 20 years and then remained stable. Parasite load was not significantly different in the two ethnic groups. For mansonelliasis, the microfilarial rate was higher in the Pygmies (67·5% compared with 22·0%) and males of the 2 groups were more frequently infected than females. Microfilarial load was also higher in Pygmies than in Bantus (mean microfilarial densities (MfD 50) 13 and 2 respectively). In the Pygmy group, MfD 50 for *M. perstans* increased with age whereas it remained stable in the Bantus. 53·8% of the 249 questioned persons had experienced worm migration under the conjunctiva. Both ethnic groups were equally exposed to the vectors of *L. loa* and reasons for the difference in prevalence of microfilaria carriers are discussed. For mansonelliasis increased contact with vectors may explain the higher degree of infestation observed in Pygmies. Other filariases were infrequent in (*Mansonella streptocerca*), or absent from (*Onchocerca volvulus* and *Wuchereria bancrofti*), the study area.

Introduction

Loa loa filariasis is found only in Africa, confined to the tropical rain forest of West and Central Africa, chiefly in the Congolese forest area: Nigeria (KERSHAW *et al.*, 1953; UDONSI, 1986), Cameroon (LANGUILLON, 1957; RIPERT *et al.*, 1977), Gabon (RICHARD-LENOBLE *et al.*, 1980; VAN HOEGAERDEN *et al.*, 1987) and Zaire (FAIN *et al.*, 1974; GRYSEELS *et al.*, 1985). *Mansonella perstans* filariasis is widespread in Africa and also occurs in intertropical America and in the south of the Caribbean Islands. Few epidemiological studies have been carried out in the Congo, which is why a world-wide expert on filariasis could write only that '*Loa loa* and *Mansonella perstans* probably occur' in the Congo (HAWKING, 1977).

Since 1982 surveys carried out in the Congo have determined the extent of filariasis with microfilaraemia (CARME *et al.*, 1986). Loaiasis is particularly endemic in primary forest and in the gradual transition zones of southern and central Congo. *M. perstans* filariasis occurs in rain forest areas in the north and the south of the country. No *Wuchereria bancrofti* cases have been detected.

We report parasitological prevalence data for these filariases in the Chaillu mountains of the Congo. Other human filariases, in particular infections caused by *Onchocerca volvulus* and *M. streptocerca*, have also been surveyed and are discussed.

Study area

Seven long-established villages in the Chaillu forest (Lekoumou region, Congo) were studied from February 1985 to February 1986. Four villages (Missama, Mapati, Loyo and Mambouana) are situated in the Sibiti district (03° 40′S;13° 20′E) and 3 (Lissengue, Massala-Moetche and Lekoli-Mouala) in the Komono district (03° 15′S;13° 15′E). Their altitude varies between 400 and 600 m and the rural density from 2 to 10 inhabitants/km^2.

The vegetation of Chaillu is essentially composed of forest-enclosed savanna. The forest is part of the great equatorial rain forest zone of Africa. It is dense, humid, evergreen and rich in flora, but the primary forest is in constant recession because of agricultural clearing and lumbering.

The climate is of Guinean forest type (southern Congolese climate). The mean annual rainfall varies between 1400 and 1600 mm including a dry season between May and August. The mean annual temperature is 22·7°C with small variations. The mean percentage relative humidity fluctuates between 80 and 90% (DENNIS & BOSSENO, 1977).

Materials and Methods

Demographic features

In each village, all volunteers over one year old were included in the present survey. Participation varied from 54% to 94% according to the village. 2313 persons were examined, of whom 1934 were from the Bantu ethnic group and 379 from the Pygmy group (Table 1). The age structure of the population survey

Table 1. Number of persons surveyed and percentage of adults in the seven villages

Village	Bantu No.	Bantu Percentage of adults[a]	Pygmy No.	Pygmy Percentage of adults[a]
Missama	302	57·3	134	64·2
Mapati	438	37·8	37	54·1
Loyo	432	52·3	130	57·7
Mambouana	340	48·5	40	55·0
Lissengue	152	73·0	19	68·4
Massala-Moetche	270	58·1	–	–
Lekoli-Mouala	–	–	19	94·7
Total	1934	51·6	379	61·7

[a]Over 20 years of age.

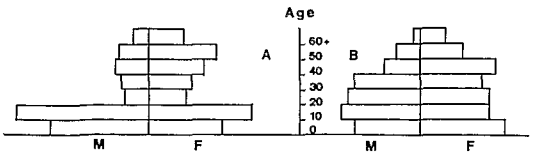

Fig. 1. Population pyramid. (A) Age (years) and sex distribution of the Bantu group surveyed (1934), compared with (B) the Pygmy group surveyed (379).

is shown in Fig. 1. The Bantu subjects included 894 males (46·2%) and 1040 females (53·8%) and the Pygmy group included 175 males and 204 females. The Bantu group were mainly subsistence farmers (cassava, banana), who supplemented their food and income by fishing, hunting and lumbering; the partially sedentary Pygmy group lived essentially by hunting.

Parasitological examination

Microfilaraemia surveys were conducted in the 7 villages. For all individuals, two 20 µl Giemsa-stained thick blood smears, collected between 1000 h and 1600 h, were examined by low power optical microscopy. Microfilariae were counted and parasitaemia was expressed as the number of parasites per 20 µl. The median microfilarial densities (MfD 50) (SASA, 1967) were calculated according to village, ethnic group, sex and age. These densities indicate the maximum number of microfilariae found per person for the least parasitized half of the infected population in this study. The results have been expressed as the nearest whole number.

Double skin biopsies were taken from the iliac crests of 211 Bantu adults from Missama (85) and Lissengue (126). The skin snips were placed in 50 µl of normal saline and 4 h later a drop of formaldehyde was added. Specimens were transported to the laboratory, where emerged microfilariae were identified and counted.

Clinical examination

One pathognomonic sign of loaiasis was looked for: adult worm migration under the conjunctiva. A random sample of adults from 3 villages (249 persons) were asked whether this symptom had occurred at least once during their life.

Results

L. loa *microfilaraemia*

Prevalence rates of *L. loa* infections are shown in Table 2. More Bantus (18·9%) than Pygmies (10·6%) were infected ($P<0·001$). The percentage of infected Bantus varied according to the village (from 13·2% in Mapati to 25·2% in Missama). However, when the values were recorded according to the mean age of population, the low prevalence rate observed in Mapati was seen to be probably related to the high percentage of patients under 20 years (62·2%). On the other hand the rate of microfilariae carriers differed significantly in Loyo and Mambouana (24·5% against 13·8%; $P<0·001$), in spite of a similar mean age. The prevalence of the infection increased with age in both sexes (Fig. 2). In the Bantu population over 30 years of age, males were more frequently infected than females ($P<0·001$), whereas under 20 years females presented a higher prevalence rate ($P<0·001$).

The highest percentages of infection in adults (age group over 20 years) were found in Loyo and Missama (respectively 40·7% and 35·8%). The youngest infected person was a girl aged 2 years.

Microfilarial densities increased until the age of 20

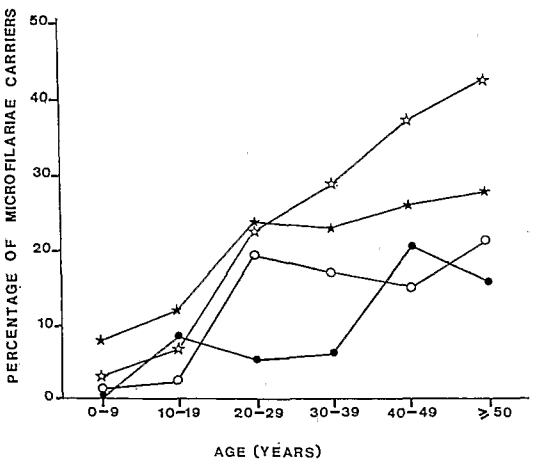

Fig.2. Prevalence of *Loa loa* microfilariae carriers by age. ☆ Bantu males; ★ Bantu females; ○ Pygmy males; ● Pygmy females.

Table 2. Prevalence of *Loa loa* microfilariae carriers and median microfilarial densities in the seven villages

Village	Bantu No.	Bantu Positive cases %	Bantu MfD 50[a]	Pygmy No.	Pygmy Positive cases %	Pygmy MfD 50[a]
Missama	76	25·2	26	18	13·4	45
Mapati	58	13·2	37	1	2·7	–
Loyo	106	24·5	16	14	10·8	14
Mambouana	47	13·8	62	0	0·0	–
Lissengue	34	22·4	40	5	26·3	3
Lekoli-Mouala	–	–	–	2	10·5	–
Moetche-Massala	44	16·3	33	–	–	–
Total	365	18·9	41	40	10·6	35

[a]MfD 50=median microfilarial density.

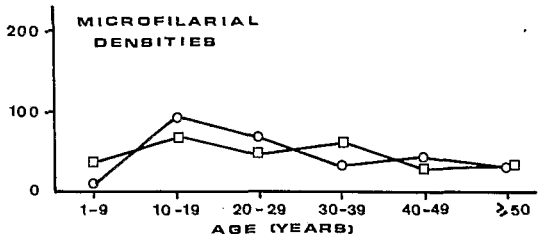

Fig. 3. *Loa loa*: trend of median microfilarial density with age. □ Bantu males and females; ○ Pygmy males and females.

Table 3. *Loa loa*: median microfilarial densities according to sex and ethnic group

		Male	Female	Male + female
Bantu	Positive cases	158	207	365
	MfD 50[a]	40	45	41
Pygmy	Positive cases	20	20	40
	MfD 50[a]	31	35	35

[a]MfD 50=median microfilarial density.

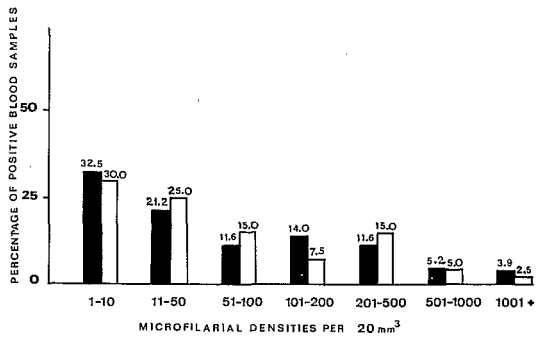

Fig. 4. *Loa loa*: distribution of microfilarial densities in subjects with positive blood samples. ■ Bantu; □ Pygmy.

years, then remained stable or even decreased (Fig. 3). No significant difference was noted between MfD 50 recorded by sex or by ethnic group (Table 3). However, the MfD 50 varied considerably according to village but without correlation with the percentage of observed infection (Table 2). The relative percentages of microfilariae carriers with regard to the densities varied inversely with the increase in parasite load (Fig. 4). The values representing the 2 ethnic groups were superimposable, plotting a linear regression curve (R^2=0·85).

Clinical evidence of loaiasis

53·8% of the 249 persons who underwent clinical examination had experienced at least one episode of a worm passing across the conjunctiva of the eye. The prevalence rate of this clinical sign was similar in the Pygmy group (56·7% of 30 questioned individuals) and the Bantu group (53·4%).

M. perstans *microfilaraemia*

The percentage of microfilariae carriers varied according to village (Table 4) and increased with age throughout life (Fig. 5). The prevalence rate of

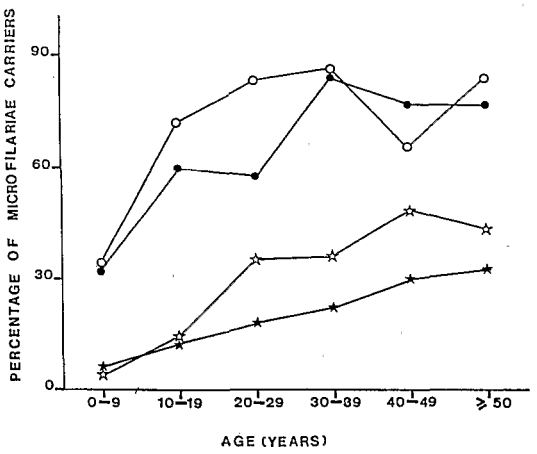

Fig. 5. Prevalence of *Mansonella perstans* microfilariae carriers by age. ☆ Bantu males; ★ Bantu females; ○ Pygmy males; ● Pygmy females.

Table 4. Prevalence of *Mansonella perstans* microfilariae carriers and median microfilarial densities in the seven villages

Village	Bantu No.	Bantu Positive cases %	Bantu MfD 50	Pygmy No.	Pygmy Positive cases %	Pygmy MfD 50
Missama	79	26·2	2	108	80·6	19
Mapati	84	19·2	2	21	56·8	11
Loyo	96	22·2	2	73	56·2	9
Mambouana	52	15·3	2	23	57·5	6
Lissengue	69	45·4	5	17	89·5	16
Lekoli-Mouala	–	–	–	14	73·7	9
Moetche-Massala	46	17·0	2	–	–	–
Total	426	22·0	2	256	67·5	13

[a]MfD 50=median microfilarial density.

Table 5. *Mansonella perstans*: MfD 50 values according to sex and ethnic group

		Male	Female	Male + Female
Bantu	Positive cases	204	222	426
	MfD 50[a]	2	2	2
Pygmy	Positive cases	123	133	256
	MfD 50[a]	13	12	13

[a]MfD 50=Median microfilarial density.

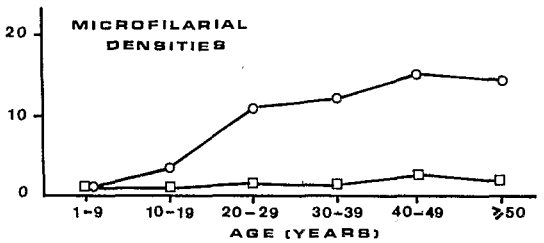

Fig. 6. *Mansonella perstans*: trend of median microfilarial density with age. □ Bantu males and females; ○ Pygmy males and females.

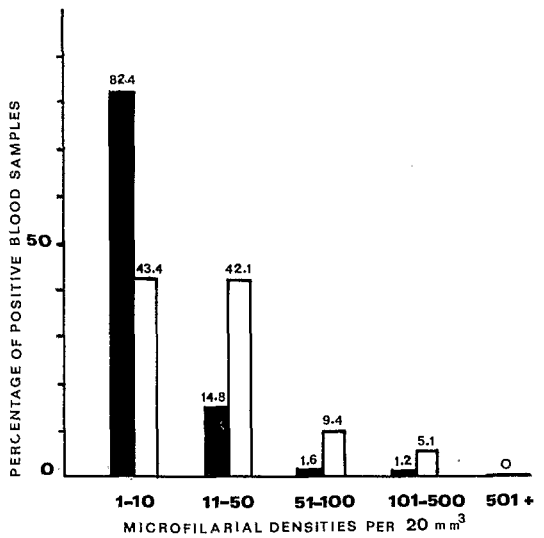

Fig. 7. *Mansonella perstans*: distribution of microfilarial densities in subjects with positive blood samples. ■ Bantu; □ Pygmy.

microfilariae in thick blood smears was significantly higher in the Pygmy group than in the Bantu (67·5% and 22·0% respectively) and was over 30% in the 0–9 years age range in the Pygmy group. In the group aged over 20 years more males were infected than females in Bantu people (42·1% compared with 28·6%; $P<0·001$).

The MfD 50 was significantly higher in the Pygmy group than in the Bantu group (Table 5). No difference was found according to sex. In contrast to loaiasis, an increase in parasitic load with age was observed in the Pygmy group (Fig. 6). There was little variation in density between the Bantu villages. However, there were marked differences between the Pygmy villages (Table 4).

The relative percentage of *M. perstans* microfilariae carriers varied inversely with the increase in parasitic load, according to an exponential regression curve ($R^2=0·93$ in the Bantus; $R^2=0·90$ in the Pygmies). 56·6% of the Pygmies but only 17·6% of the Bantus had a microfilarial density equal to or higher than 11 (Fig. 7).

Prevalence of mixed infections

The ethnic groups and sex-related distribution of mixed infections (*L. loa* and *M. perstans*) are summarized in Table 6. The expected percentage of mixed infections (prevalence rate of *L. loa* microfilariae carriers also infected with *M. perstans*, theoretically identical to that of the general population) was not significantly different from the observed percentage in the Pygmy group but it was twice the observed percentage in the Bantu. The presence of both microfilarial species in one subject did not seem to affect the parasite load. Thus, in the Bantus, the MfD 50 were 48 and 3 for *L. loa* and *M. perstans* respectively for single infections, and 51 and 3 for mixed infections.

Prevalence of other filariases

Only 3 subjects had *O. volvulus* microfilariae in the iliac crest snips (1·4%). Filariasis due to *M. streptocerca* was found only in Komono district (Lissengue), where 9 subjects were infected (7·1%). No *W. bancrofti* infection was found.

Discussion

In the Chaillu area, loiasis is the third most common reason for attending rural hospitals (BOULESTEIX & CARME, 1986) on account of the physical and psychological repercussions. The medical and economic consequences of this filariasis have

Table 6. Prevalence of mixed infections: comparison of the observed and theoretical rates

	No.	*L. loa*%	*M. perstans*%	Mixed infections Observed %	Mixed infections Theoretical expected %	Ratio of observed theoretical expected
Bantu						
Male	894	17·7	22·8	8·9±1·9	4·0±1·3	2·2
Female	1040	19·9	21·3	8·4±1·7	4·2±1·2	2
Pygmy						
Male	175	11·4	70·3	10·3±4·5	8·0±4·0	1·3
Female	204	9·8	65·2	6·9±3·5	6·4±3·4	1·1

thus been under-estimated. The high prevalence of loaiasis in Chaillu is related to the ecology of this area of Central Africa (the humid mountain forest), which encourages the breeding of *Chrysops* (RICHARD-LENOBLE *et al.*, 1980).

FAIN (1978) reported that the prevalence rate of adults presenting with microfilaraemia was always less than 35%. In the village of Loyo we found a rate of over 40%.

We have shown that males over 30 years of age were more infected than females. This confirms other results obtained from Zaire (FAIN *et al.*, 1974), Cameroon (RIPERT *et al.*, 1977), and Gabon (VAN HOEGAERDEN *et al.*, 1987; LANGUILLAT *et al.*, 1978).

The lower infection rate observed in women could be due to sex-related genetic factors (VANHOEGAERDEN *et al.*, 1987) or to an endocrine factor (NELSON *et al.*, 1962). The high prevalence rate observed in females under 20 years does not support these hypotheses however, but this may be related to differences in exposure of children and adults. Pygmies, dwellings are situated at the end of Bantu villages. The densities and infection rates of *Chrysops* were the same in all parts of the villages studied (F. Noireau, unpublished observations), indicating similar exposure to the vector for both ethnic groups. Although the prevalence rate of *L. loa* microfilaraemia was lower in the Pygmy community, the overall prevalence rate of loaiasis was probably similar in both ethnic groups, as suggested by the similar frequency of worm migration under the conjunctiva and the similar distribution of microfilarial densities. A survey conducted in 1962 in the Pygmies of Zaire (MANN *et al.*, 1962) found that *L. loa* microfilariae carriers were extremely rare (1 positive case out of 256 subjects). PAMPIGLIONE *et al.* (1979) found 10·3% microfilariae carriers among Pygmies of east Zaire in 1971 and 69·5% in 1972. The results of these two surveys conducted at a one-year interval on the same Pygmy group seem to be completely contradictory.

The lower number of microfilariae carriers in the Pygmy group does not seem to be related to environmental factors (RAVISSE, 1955) but might be due to a protective immune response against the *L. loa* adult worms and microfilariae (PINDER, 1988).

The genetic predisposition to the expression of microfilaraemia suggested in 1955 (GORDON, 1955) has not been confirmed. No correlation has been shown between blood groups and *L. loa* microfilariae carriage (OGUNDA, 1970). No research on the HLA system and loaiasis has been carried out, but studies on lymphatic filariasis have revealed a familial predisposition to the infection which is not related to HLA specificity (OTTESEN *et al.*, 1981). In contrast to *W. bancrofti* filariasis (WEIL *et al.*, 1983), no prenatal sensitization to *L. loa* and *M. perstans* microfilarial antigens has been demonstrated (VAN HOEGAERDEN & AKUE, 1986).

It is most probable that subjects living in highly endemic zones are repeatedly inoculated with *L. loa* infective larvae (L3). The paradoxical decrease in microfilarial density observed during life might be explained by the sterilization of the female worms or by increasing microfilaricidal immunity, giving partial or total control of microfilaraemia. Thus, in the monkey, no correlation has been found between the intensity of microfilaraemia and the number of female worms (ORIHEL & EBERHARD, 1985). In addition, the demonstration of higher antibody levels in individuals without detectable microfilaraemia (PINDER, 1988) suggests that protective immunity varies among a population subjected to similar exposure to infective larvae. Another possibility might be the development of immunity against the L3 or L4 stages (WHO, 1984). In this case only the females from former infections would ensure egg-laying, given the longevity of adult *L. loa* (COUTELEN, 1935).

Pygmies, who are more exposed than Bantus to *Culicoides grahami* bites during their long stays in the forest, present a higher prevalence of individuals infected with *M. perstans* from childhood and significantly higher microfilarial densities. The acquisition of protective immunity may produce only a relative decrease in microfilaraemia, as suggested by infections of nearly 100% observed in certain surveys (GORDON *et al.*, 1950). The finding that densities increase with age supports this hypothesis and confirms the differences between the immune mechanisms involved in these two filariases, whereas they had previously been considered to be similar (ORLANDO *et al.*, 1982).

The rarity, in the study area, of anthropophilic black-flies (*Simulium* spp.) accounts for the absence of local transmission of onchocerciasis. The low prevalence rate observed for *M. streptocerca* filariasis implies that the cases were imported; this is in spite of the presence of a theoretically effective vector (DUKE, 1954).

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Announcements

Short course in advanced epidemiological methods

This intensive course will be held at the London School of Hygiene and Tropical Medicine, University of London, from 11–22 September 1989. It is intended for those who already have a working knowledge of basic epidemiology and statistics, although a strong mathematical background is not necessary. The course fee is £800.

Course brochure and application form available from: The Registrar, London School of Hygiene and Tropical Medicine, Keppel Street, London, WC1E 7HT, England.

Eighth European course in tropical epidemiology

20th August to 2 September 1989, Liverpool, England

This course is open to physicians and other health workers and researchers concerned with epidemiology in developing countries.

Further information is available from David Stevenson, Department of International Community Health, Liverpool School of Tropical Medicine, Pembroke Place, Liverpool, L3 5QA, UK.