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Comparison of thick blood smear and saponin haemolysis for the detection of *Loa loa* and *Mansonella perstans* infections

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Summary

In an endemic area, *Loa loa* and *Mansonella perstans* microfilariae were detected by the examination of 5 ml blood in respectively 7.4 and 26.2% of subjects who would have been erroneously considered amicrofilaraemic by the conventional method of two thick blood smears (40 µl blood). Correction factors to be applied to the results obtained with thick blood films in order to approach the true parasitological prevalences were 1.5 for *L. loa* and 1.6 for *M. perstans*. In addition, the analysis of a large volume of blood provides a better estimation of microfilarial load of the parasitized population.

Introduction

The epidemiological evaluation of filarial infection relies on clinical, parasitological, immunodiagnostic and entomological methods (WHO 1984). In *Loa loa* and *Mansonella perstans* filariases, parasitological data take into consideration the prevalence and intensity of microfilaraemia and measure different aspects of the endemicity of the parasites in the population (Noireau *et al.* 1989). In the field, the search for microfilariae (mf) is usually carried out by examination of thick blood smears prepared from specimens of capillary blood (Sasa 1967). However, a greater volume of blood, obtained by venipuncture, is necessary to detect low-density carriers. The concentration of mf by sedimentation of haemolysed blood was first described by Knott (1939). The most commonly used enriching techniques are saponin haemolysis (Franks & Stoll 1945), membrane filtration (Bell 1967; Dennis & Kean 1971) and density gradient centrifugation (Jones *et al.* 1975). Comparative studies of parasitological techniques conducted in a region where loaiasis is endemic are scarce (Gordon & Weber 1955; Richard-Lenoble *et al.* 1980) although it has been demonstrated in foci of lymphatic filariasis that the use of a concentration technique increases three- to five-fold the chances of detecting mf (Ramachandran 1975). We estimated the mf prevalence rate and microfilarial density in a population sample living in an area endemic for *Loa loa* and *Mansonella perstans* by examining thick blood films, and the results were compared with data obtained with an enrichment technique such as haemolysis/centrifugation. Haemolysis was chosen as the reference test rather than filtration since it was found to be more sensitive in that a greater volume of blood could be examined (Richard-Lenoble *et al.* 1980).

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Materials and methods

Capillary and venous blood samples were taken from 201 adult Bantus (mean age: 35.9 ± 13.2 years; M:F sex ratio: 1.2:1) between 1000 and 1400 h. Two 20-µl thick blood films were prepared from each sample of capillary blood. After Giemsa staining, the mf were identified and counted. Citrated venous blood (5.0 ml) was lysed with 2% saponin. The mf in the sediment after centrifugation were identified and counted after Giemsa staining.

Table. *Loa loa* and *Mansonella perstans*: comparison of two parasitological techniques for the estimation of prevalences of microfilariae carriers and microfilarial densities

Filariae identified	No. samples	Thick smear			Haemolysis		
		+	+%	MfD	+	+%	MfD
L. loa	201	26	12.9	550.0	39	19.4	135.5
M. perstans	201	60	29.9	106.9	97	48.3	42.2

MfD, Geometric mean of microfilarial densities per ml of blood.
Thick smear, 40 µl of capillary blood.
Haemolysis, 5 ml of venous blood.

The value of thick blood smears in the detection of both species of mf was studied by determining its sensitivity (or rate of co-positivity) and its negative predictive value. The correction factors to be applied to the results obtained with thick blood films in order to approach the true parasitological prevalences were calculated by dividing the number of positives observed in the examination of 5 ml of blood by the number of positive cases to be found by examination of 40 µl (Sasa 1967).

For both types of samples, the microfilarial load of the parasitized population was expressed as the geometric mean of microfilarial densities (MfD) per millilitre of blood.

Results and discussion

The results are shown in Table 1. Twenty-six subjects showed *L. loa* mf in the thick blood films and 39 in the haemolysis test (60 *vs* 97 for *M. perstans*). The rate of co-positivity was 0.67 for *L. loa* and 0.62 for *M. perstans* whereas the negative predictive values were respectively 0.93 and 0.74. All negative results obtained with haemolysis were also negative with the thick smears. The correction factors to be applied to the number of cases positive with the thick smears were 1.5 for *L. loa* and 1.6 for *M. perstans*. Above the detection threshold with thick blood film (> one mf per 40 µl of blood), the quantitative distributions of *L. loa* and *M. perstans* microfilaraemia were similar with both techniques. For *L. loa*, the average microfilarial density calculated by thick blood films was 4 times that observed after haemolysis, which included low-density carriers (550.0 *vs* 135.5 mf ml^{-1}). For mansonellosis, the average microfilarial densities were 106.9 mf ml^{-1} (thick smear) and 42.2 mf ml^{-1} (haemolysis).

The evaluation of the prevalence of mf carriers is the basis of studies on human filariases (Sasa 1967), particularly filariasis due to *L. loa* and *M. perstans* (Kershaw 1950). For infection with *M. perstans* the pathogenicity of which is not clearly established (Janssens 1964; Wiseman 1967), there are no reliable clinical manifestations which can be taken into consideration. However, for loaiasis, the prevalence of a pathognomonic symptom such as subconjunctival migration of the adult worm is an interesting epidemiological indicator which complements the estimation based on mf carriers (Noireau *et al.* 1990). The immunodiagnosis of *L. loa* filariasis enables the immunity of the amicrofilaraemic population to be assessed (Pinder 1988).

In our study, the estimation using negative predictive values indicated that respectively 7.4 and 26.2% of the subjects would have been erroneously considered negative for *L. loa* and *M. perstans* by the examination of only 40 µl of blood. The correction factors applicable to results obtained with thick blood smears ($\times 1.5$ for *L. loa* and $\times 1.6$ for *M. perstans*) are similar to those determined in other regions of Central Africa. Indeed, Richard-Lenoble *et al.* (1980) defined a coefficient for microfilaraemia filariasis in Gabon as 1.33 and Gordon and Weber (1955) estimated it as 1.6 in Cameroon. Consequently, although these values depend on the distribution of microfilarial densities, and to a lesser extent the rate of infection within the population (Sasa 1967), they can however be considered as relatively constant in the whole of the forest region of Central Africa where these two filariases are endemic. An enrichment technique such as haemolysis enables populations with low parasitic loads to be taken into account for the calculation of microfilarial densities. In addition, correction coefficients to be applied to the density results obtained with thick blood films can be determined (a fourfold decrease for *L. loa* and 2.5-fold for *M. perstans*). Although it is not essential to take these coefficients into account in epidemiological studies, they can in

fact be applied since it has been confirmed that there is no significant difference between the number of mf in the same volume of venous and capillary blood (Gordon & Weber 1955).

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