Loa loa Microfilarial Periodicity in Ivermectin-Treated Patients: Comparison Between Those Developing and Those Free of Serious Adverse Events

Joseph Kamgno,* Sébastien D. Pion, Charles D. Mackenzie, Björn Thylefors, and Michel Boussinesq

Filariasis Research Centre and Faculty of Medicine and Biomedical Sciences, University of Yaounde I, Yaounde, Cameroon;
Unité Mixte de Recherche 145, Institut de Recherche pour le Développement and Université Montpellier 1, Montpellier, France;
Filarial Diseases Unit, Michigan State University, East Lansing, Michigan; Mectizan Donation Program, Decatur, Georgia

Abstract.   The main risk factor of post-ivermectin serious adverse events (SAEs) is the presence of a high *Loa loa* microfilaremia. However, the majority of patients with such high loads do not develop SAEs, suggesting that co-factors may be involved. An infection with simian *Loa* parasites, whose microfilariae show a nocturnal periodicity, might be such a co-factor. The periodicity of *Loa* microfilariae was compared, using cosinor methodology, in 4 patients who had developed a post-ivermectin neurologic SAE, 4 patients who had experienced a non-neurologic SAE, and 14 control individuals. The periodicity was similar in all three groups, with a peak of microfilaremia occurring between 12:30 and 2:00 PM. The results of this study, which for the first time characterizes the periodicity of *Loa* microfilariae mathematically, suggest that post-ivermectin SAEs are not related to an infection with a *Loa* simian strain.

INTRODUCTION

Over the past 17 years, serious adverse events (SAEs) have been recorded after treatment of onchocerciasis with ivermectin in a number of individuals living in *Loa loa*–endemic areas.[1–3] It has been shown that the risk of developing such SAEs is strongly related to the individual *L. loa* microfilarial loads.[4] Marked reactions (including functional impairment for several days, requiring assistance in performing normal daily functions and household activities) and SAEs (accompanied by functional impairment requiring at least 1 week full-time assistance and sometimes by problems of consciousness) may occur when the microfilaremia exceeds 8,000 and 30,000 microfilariae (mf)/mL, respectively. However, the vast majority of patients harboring a very high *L. loa* microfilaremia do not develop a SAE after ivermectin treatment. In communities highly endemic for loiasis located in central Cameroon, the proportion of individuals harboring > 30,000 mf/mL can reach 6%,[5] whereas in the same area, the incidence of overall SAEs and of encephalopathic SAEs was found to be, respectively, 2.7 and 1.9 per 10,000 patients treated.[6] These observations suggest that, aside from a high *L. loa* microfilarial load, there may be other factors related to either the patients or the parasite that could explain why some individuals develop adverse reactions after treatment with ivermectin, whereas others do not.

The pathogenic mechanisms associated with *Loa*-related post-ivermectin SAEs are not well known. The appearance of the retinal hemorrhages seen in patients who have developed such a SAE,[7] and the results obtained in primates experimentally infected with *L. loa* (S. Wanji and C. D. Mackenzie, unpublished data), suggest that the condition might be related to an obstructive process in the cerebral microcirculation and perhaps elsewhere in the body. However, the possibility that SAEs are at least partially caused by the passage of *L. loa* mf into the central nervous system (CNS) cannot be ruled out. After treatment with diethylcarbamazine (DEC), *Onchocerca volvulus* mf migrate to the blood, the urine, and the cerebrospinal fluid (CSF).[8,9] The passage of *O. volvulus* mf into the blood and/or the urine has also been reported after treatment with albendazole[10] and ivermectin.[11–13] The presence of *L. loa* mf in the CSF of patients who have developed a SAE after

DEC treatment[14] and the fact that *L. loa* mf were found in the brain parenchyma in one of these cases[15] may indicate that DEC induces a migration of *Loa* mf in the CNS. Such a phenomenon has been clearly shown in loiasis patients for whom CSF samples have been collected before and after ivermectin treatment.[1] Given this, it is possible that the pattern of mf mobilization may vary between different strains of a given filarial species. Thus, it seems that *O. volvulus* mf belonging to the savanna strain are much more able to migrate into the CSF after DEC treatment[8] and into the urine after injection of DT TAB vaccine (used as a pyrogenic factor)[16] than mf of the forest strain. This might be because of differences in pre-treatment location of mf[17] or differences in their ability to cross some barriers. Differences in the distribution of mf within the human host, and in the capacities of mf to leave the bloodstream to invade other milieus, including the CNS, may also exist for *L. loa*, a parasite for which it is known that there are at least two strains: one that parasitizes humans and the other that naturally infects various species of monkeys.[18] Such differences in strain might also be reflected in the severity of the host response to mf with humans reacting more strongly to the animal strain than to its own parasite.

The two strains of *L. loa* differ in their daily microfilarial periodicity. Maximum *L. loa* microfilarial density in the peripheral bloodstream is observed during daytime with the human strain, whereas the peak of microfilaremia occurs during the night with the simian strain.[18] The vectors of the human strain (*Chrysops silacea* and *C. dimidiata*) bite during the day, whereas those of the simian strain (principally *C. langi* and *C. centurionis*) are active during the night.[19] Duke[20] showed that the human strain of *L. loa* easily develops in various species of monkeys in which the two strains can hybridize. The mf resulting from these inter-breedings show a complex periodicity.[20] Although the only attempt to infect a human with simian *Loa* adult worms was unsuccessful,[21] the possibility of human infection with a simian strain cannot be ignored. During a survey conducted in the Bas-Congo Province of the Democratic Republic of Congo (DRC), Fain and others[22] collected blood samples from > 2,000 individuals by day (11:00 AM) and by night (9:00–11:00 PM). Among the 535 persons who had *Loa* mf present in at least one sampling, 16 showed parasites only in the night sample (with fairly low loads), 10 showed more mf in the night sample than in the day sample, and 62 showed "almost as many mf in the night sample as in the day sample."[22]

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* Address correspondence to Joseph Kamgno, Filariasis Research Centre, BP 5261, Yaoundé, Cameroon. E-mail: jkamgno@yahoo.fr

This study was based on the hypothesis that infection with parasites of animal origin may be involved in the pathogenesis of SAEs occurring after ivermectin treatment. Taking advantage of the fact that an infection with a simian *L. loa* strain can be detected by analyzing the pattern of microfilarial periodicity, we compared the changes in the microfilarial loads during a 24-hour period in patients who developed post-ivermectin SAEs with subjects who did not. In addition, this study constitutes the first attempt to characterize mathematically the periodicity of *L. loa* mf.

MATERIALS AND METHODS

Study site. The study was conducted in the Bankim health district in the Adamaoua Region of Cameroon. The vegetation is essentially of savanna type with forested areas favorable to the presence of the vectors of *L. loa*. This area is hyperendemic for both onchocerciasis and loiasis, with prevalence of *Loa* microfilaremia in the total population exceeding 35% in some villages.[23]

Selection of patients. In 2003, after the campaign of annual Community-Directed Treatment with Ivermectin (CDTI), 11 cases of SAEs, including a fatal case, were recorded in six villages of the Bankim health district (Kimi Pettel, Kouroum, Mbiridjom, Sarki Barka, Songkolong, and Tchim). These SAEs cases were followed and fully examined during their SAE episode by J.K., who ascertained that the condition experienced by the patients met with the case definition of SAEs proposed by the Mectizan Donation Program.[3] Six months later, a parasitologic survey was conducted in the same communities to determine the *Loa* microfilarial loads of those 10 patients who had survived their SAE. The microfilarial loads of 106 subjects living in the same communities and who had not developed any reaction after treatment were also measured to identify persons who could be enrolled as controls for the study. The treatment history of these potential controls was verified from the registers filled by the community distributors.

Two of the 10 surviving patients experiencing SAEs were absent from their village at the time of the survey and could not be examined. Among the eight remaining cases, four had developed an encephalopathy with problems of consciousness and objective neurologic signs, and four had experienced serious functional impairment without troubles of consciousness (i.e., a non-neurologic SAE).[4] Potential controls were selected by matching with SAE cases for sex, age (with differences between the case and control subjects being as much as possible < 5 years), village of residence, and *L. loa* microfilarial load. The three former factors were chosen to ensure similar possible exposition to simian *Loa* strain between cases and controls. All these individuals were given a detailed explanation of the objective and procedures of the study, with emphasis given to the fact that blood samples would be taken repeatedly within a period of 24 hours.

All the 8 SAE cases, and 14 of the 15 persons who were found eligible as controls, accepted to participate and signed an informed consent form. All subjects were given a general medical consultation and hospitalized at the district hospital in Bankim for 1 full day. Because of the limited number of beds in the hospital, the study lasted for 2 days, with 12 individuals examined on the first day and 10 on the second day.

Collection of blood. Blood samples were first taken from each individual at 6:00 PM and then at 8:00 PM, 10:00 PM,

12:00 AM, 2:00 AM, 4:00 AM, 7:00 AM, 10:00 AM, 12:00 PM, and 3:00 PM. Because of the high number of procedures taking place, the samples were collected within an interval of 30 minutes, starting 15 minutes before the designated hour and ending 15–20 minutes after that hour. The patients were discharged at 4:00 PM and driven back to their respective communities. It has been shown that the density of *Loa* mf in the peripheral blood is very sensitive to body temperature[24]; thus, all subjects were hospitalized in the same large room at ambient temperature to avoid a possible influence of this factor on the microfilarial counts.

Calibrated blood smears for the quantitative examination of *Loa loa.* Microfilarial densities were quantified using calibrated blood smears. Blood was collected by fingerprick using a sterile lancet and collected in non-heparinized capillary tubes. A volume of 50 µL of blood was spread on a slide, dried at room temperature, and stained with Giemsa stain within 6–24 hours of sampling. *L. loa* and *Mansonella perstans* mf were counted under a microscope using the magnitude 100.

Data analysis. The cosinor model,[25] commonly used to study variables governed by circadian or other biological rhythms, was applied to detect the presence of, and describe any diurnal pattern in, *Loa* microfilaremia. This method entails fitting an oscillating curve to temporal dynamic variables using a specified (e.g. 24-hour) periodicity. The cosinor model can be expressed as $y(t) = M + A \cos \varphi \cos \omega t - A \sin \varphi \sin \omega t$, where y represents the observed microfilaremia and t represents time of observation. The constant $\omega = 2\pi/24$ represents the 24-hour periodicity of the *Loa* microfilaremia. The coefficient M represents the 24-hour rhythm-adjusted mean (MESOR) defined as the average value of microfilaremia. Parameters A and φ, respectively, represent the amplitude (defined as one half the widest variation in microfilaremia within a 24-hour period) and the acrophase (determining the time of the peak) of the cosinor model. The cosinor model was adjusted, using the least square method, to individual data in each separate group: the control group, the non-neurologic SAE group, and the neurologic SAE group. The values of the phase parameter φ were compared between the three groups.

Ethical approval. The study protocol was approved by the National Ethics Committee of Cameroon.

RESULTS

Patient characteristics. The mean ages of the four patients who had developed encephalopathy and that of the four subjects who had experienced an SAE without troubles of consciousness were 31 years for both groups (ranges: 20–53 and 20–39, respectively). The mean *L. loa* microfilaremia during the SAE episode was 1,545 mf/mL (range: 1,320–1,840) in patients who developed a neurologic SAE and 1,125 mf/mL (range: 1,020–1,320) in those who had a non-neurologic SAE. In these two groups, the first symptoms occurred on average 1.0 and 1.25 days after treatment with ivermectin, and the mean hospitalization durations were 15 and 5.75 days, respectively. In the control group, the mean age was 35 years (range: 16–60). Among the 22 subjects included in the study, 19 were men and 3 (1 in each group) were women.

Microfilarial loads over a 24-hour period in the three groups. Only two patients, one non-neurologic case and one control subject, harbored *M. perstans* mf, with loads ranging from 15 to

28 and 1 to 3 mf/50 µL, respectively. No *Wuchereria bancrofti* mf was found in any sample.

In the three groups, the lowest individual *Loa* microfilaremias were recorded during nighttime (Figure 1A) and the highest values were between 10:00 AM and 6:00 PM (Figure 1B). At midnight, mf were detected in the peripheral blood of two neurologic SAE cases, three non-neurologic SAE cases, and six controls. The individual amplitudes ranged from 55 to 755 mf/50 µL in the neurologic SAEs group, from 93 to 183 mf/50 µL in the non-neurologic SAEs group, and from 12 to 471 mf/50 µL in the control group. There were slightly different trends in the afternoon between the groups, with a slower decline in the mean loads in the control group and in the group of patients who had developed non-neurologic SAEs. In these two groups, three and two subjects, respectively, showed their highest microfilaremia at 6:00 PM.

Figure 2 shows the observed and predicted mean *Loa* microfilaremias for the three groups over a 24-hour period. The cosinor model provided adequate fits to individual data in each group. In the neurologic SAEs group, the amplitude A and acrophase φ were, respectively, 132.2 and –0.16, the latter value corresponding to a peak of microfilaremia occurring at 12:37. In the non-neurologic SAEs group, A and φ were, respectively, 67.0 and –0.54, corresponding to a peak of microfilaremia at 14:04. In the control group, A and φ were respectively 66.0 and –0.27, corresponding to a peak of microfilaremia at 13:02. The 95% CIs for φ in the three groups were, respectively, [–0.86; 0.53], [–0.86; –0.22], and [–0.66; 0.12], indicating no statistical difference in periodicity between the three groups.

DISCUSSION

Periodicity of mf in the peripheral blood is a common characteristic among filarial parasites of humans and other animals.[26] When mf are not circulating in the peripheral blood, they are thought to be in the small vessels of the lungs.[24,26] The pattern of periodicity shown by the mf produced after interbreeding of human and simian strains of *L. loa*,[20] as well as mathematical analyses of data collected in various populations of *W. bancrofti*,[27] indicate that the characteristics of periodicity are genetically determined. However, the circadian rhythm of the host also plays a role. Thus, Hawking,[28] using his own observations and those obtained by Kershaw,[29] showed that peak microfilaremias in nine prisoners who woke up and went to bed very early occurred about 4 hours before those observed in seven hospital patients who had the daily sleep habits of the general population.

To the best of our knowledge, only four studies have been carried out on humans to assess the periodicity of *L. loa*; this is probably because, at least in part, of the difficulty of collecting repeated blood samples within a 24-hour period from a single individual. Besides the two studies mentioned in the preceding paragraph,[28,29] a study involving four patients was conducted in Nigeria; however, the blood samples were taken only between 8:00 AM and 10:00 PM.[30] The most recent study, performed in the Republic of Congo, included five subjects followed between 6:30 AM and 6:00 PM.[31] Thus, and taking into account the fact that the seven patients examined by Hawking were sampled only four to five times between 8:00 AM and 10:00 PM,[28] it seems that this study is the first in which individuals belonging to the general population have been examined regularly during a complete 24-hour period.

Since the pioneering works of Sasa and Takaka,[32] many analyses have been conducted to characterize mathematically the periodicity of mf. These studies have been carried out on a variety of human and animal filariae, but principally with *W. bancrofti*, *Brugia malayi,* and *Dirofilaria immitis*.[33–38] Generally, the analyses assume that the periodicity follows a harmonic wave pattern and are performed using the trigonometric method developed by Aikat and Das.[39] In this study, which is the first in which the periodicity of *L. loa* mf is analyzed mathematically, we used the cosinor method. The latter is similar to that of Aikat and Das, but it is simpler and can deal with non equidistant data (as it is the case in the present dataset). Cosinor analyses have been used in parasitology to characterize, for example, the pattern of cercarial emergence of *Schistosoma mansoni* from its intermediate host[40] and the periodicity of *W. bancrofti* mf.[41]

Although rare, the possible occurrence of post-ivermectin *Loa*-related SAEs constitutes a major impediment in
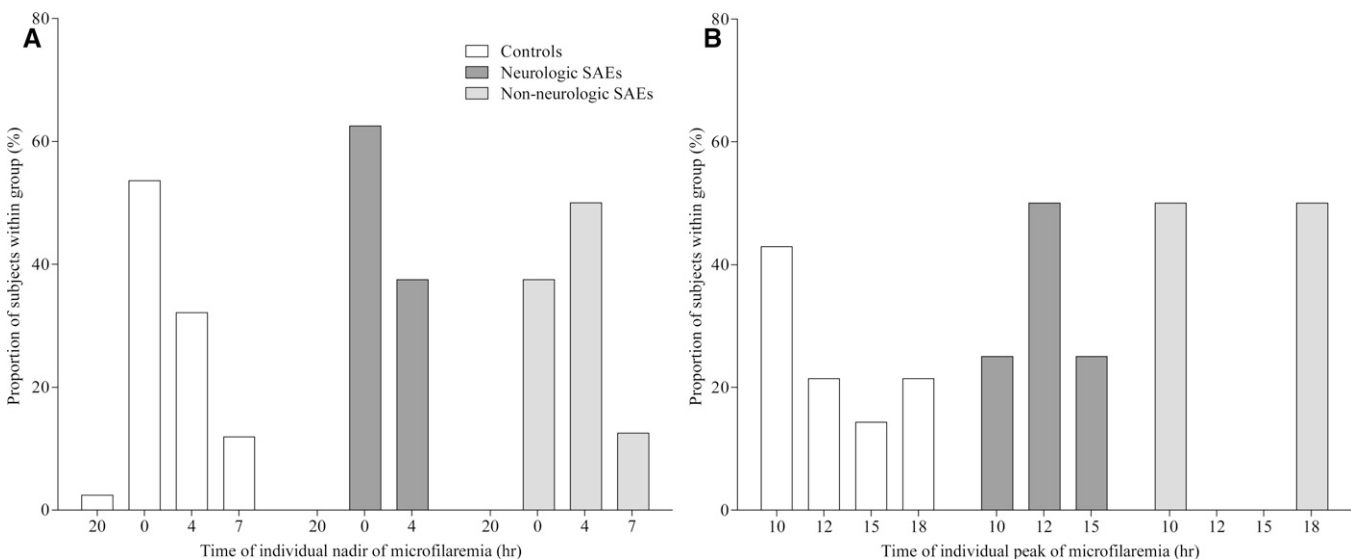

FIGURE 1. Distribution of study subjects according to the time of their lowest value (A) and peak (B) of microfilaremia in the different groups.
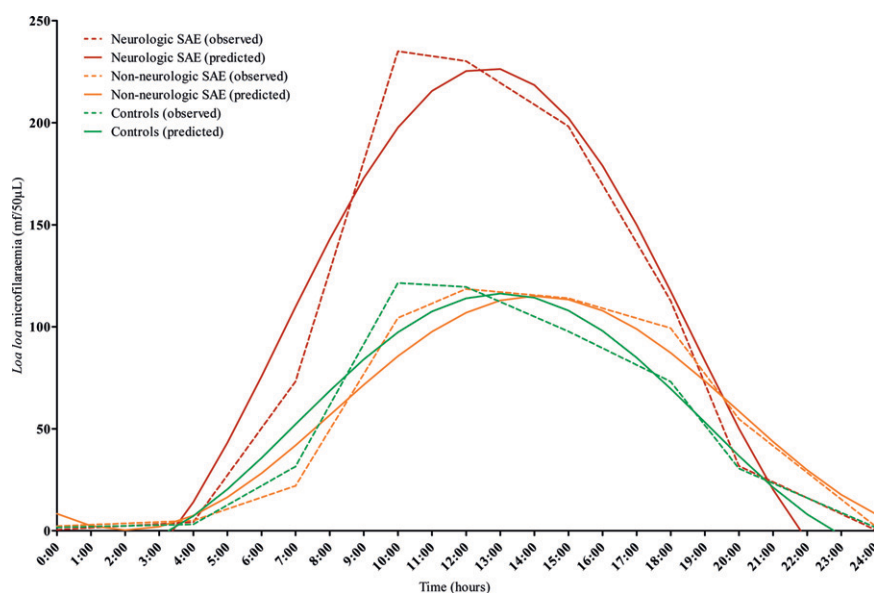
FIGURE 2. Profile of the *L. loa* periodicity in SAE cases and control subjects: observed microfilaremia (dashed lines) and mean microfilaremia predicted using the Cosinor model (solid lines).

the development of CDTI against onchocerciasis in Central Africa.[42] In addition, it delays the launching of control programs against lymphatic filariasis (LF) in those areas where onchocerciasis is non endemic but where loiasis is endemic.[43] In these regions, the strategy is to reduce and interrupt the transmission of *W. bancrofti* through mass treatment with ivermectin and albendazole. Because the treatment itself is not believed to have any effect on the overt clinical manifestations of LF, and because these individuals, not being infected by *O. volvulus*, would not benefit from the effects of ivermectin on the manifestations of onchocerciasis, it is unwise to subject them to the risk of developing a SAE. By identifying a co-factor facilitating the occurrence of SAEs, it might be possible to identify those individuals who are particularly at risk and, after having discarded them from mass treatment, to safely implement ivermectin distribution in the rest of the population.

Development of SAEs may also be related to host genetic factors; however, SAEs have been reported from geographically distant countries (principally Cameroon, DRC, and Sudan),[3] and no familial clustering has ever been described. Co-infections with *Mansonella perstans*,[4] *Plasmodium* spp. (J. Kamgno and others, unpublished data), or *Trypanosoma* spp. (M. Boussinesq and others, unpublished data) do not seem to increase the risk of SAE. The possibility that SAEs are related to the presence of simian or hybrid *Loa* strains in the human host was proposed by Duke.[44] *L. loa* has been found in several species of monkeys, with prevalences reaching 96% in the drill (*Mandrillus leucophaeus*).[18] Given the different pattern of microfilarial periodicity in the human and simian *L. loa* and the different biting habits of the vectors of the two forms, natural infection of humans with simian parasites is probably rare.[20] However, a proportion of people living in the endemic areas are still active outside their houses at dusk and the first hours of the night and can certainly be bitten by *C. langi* and *C. centurionis* and therefore be infected by the simian *Loa*. Besides this, the human pressure on the known simian natural hosts of *Loa*, particularly *M. leucophaeus* and *Cercopithecus nictitans martini*, has probably reduced significantly the res-

ervoir of simian *Loa* during the last decades. However, other hosts, such as *Cercopithecus mona mona*, are still abundant and thus the possibility that simian *Loa* occasionally infects humans cannot be discarded.

This study is the first to compare a phenotypic characteristic of the *Loa* parasites harbored by patients who developed a post-ivermectin SAE and by controls. The pattern of periodicity was found to be similar in all three groups of patients, with a peak of microfilaremia occurring between 12:00 and 2:00 PM, and thus it is unlikely that the presence of simian *Loa* parasites is a factor influencing the pathogenesis of this condition. However, the fact that similar periodicities were seen in all the groups 6 months after treatment does not exclude the possibility that some patients may have harbored simian *Loa* at the time of treatment. This would be possible if the latter strain is more sensitive to some effects of ivermectin than the human strain. Strain variation in response to various drugs has been studied or considered for *O. volvulus*,[45–47] but no definitive conclusion has been drawn. The issue is even more difficult to address for *L. loa*, because of the paucity of information on the effects of ivermectin on the adult stage of this parasite. Ivermectin given at a dose of 150–200 μg/kg of body weight provokes a sharp decrease in the *Loa* microfilaremia within the first days after treatment.[1,48,49] Then, the microfilarial loads seem to remain more or less stable at low levels (< 20% of the initial load) for at least 1 year.[50,51] This long-standing effect is probably because of an interruption of the release of new mf by the adult females, as is the case for *O. volvulus*. However, because of the difficulty in collecting *Loa* adult worms, this hypothesis has still to be proven. In addition, no follow-up has been made after 1 year in any study, and thus the duration of this so-called "embryostatic effect" is still poorly documented. In addition, no trial has ever been conducted on the effect of ivermectin on simian *L. loa*. Thus, it is difficult to compare the effects of the drug on the various strains of the parasite. However, one may assume that, even if any difference in sensitivity exists, it would not be so marked that no mf of simian origin would be circulating 6 months after treatment.

This study seems to eliminate one possibility for the as-yet-unknown cofactors for developing SAE after ivermectin: the presence of a simian strain of *L. loa*. Further studies, including the genetic analysis of *Loa* worms isolated from different clinical groups and controls, could provide additional information. In a previous study, DNA sequences of potentially polymorphic loci were compared among *L. loa* parasites from southern Cameroon and other endemic foci in sub-Saharan Africa. Three genes were analyzed: the mitochondrial 16S rRNA gene, the ITS2 domain of the nuclear rRNA gene cluster, and the 15r3 polyprotein gene.[52] These genes were almost similar for strains from Cameroon and from infected expatriate former residents of Nigeria, Gabon, and DRC. This study did not support the hypothesis that *L. loa* parasites from southern Cameroon represent a genetically isolated population. However, the parasites were not isolated from patients who had experienced a SAE, and the number of samples tested was small. More detailed analyses of *L. loa* strains from SAE cases, other individuals, as well as monkeys, may be useful.

Authors' addresses: Joseph Kamgno, Filariasis Research Centre, BP 5261, Yaoundé, Cameroon, Tel: 237-22202442, Fax: 237-22202443, E-mail: jkamgno@yahoo.fr. Sébastien D. Pion, Unité Mixte de Recherche 145, Institut de Recherche pour le Développement, 911 Avenue Agropolis, BP 64501, 34394 Montpellier Cedex 5, France, Tel: 33-467416148, E-mail: Sebastien.Pion@ird.fr. Charles D. Mackenzie, Filarial Diseases Unit, Michigan State University, A54 VMC, East Lansing, MI 48824, Tel: 517-432-3644, E-mail: mackenz8@msu.edu. Björn Thylefors, Mectizan Donation Program, 325 Swanton Way, Decatur, GA 30030, E-mail: bthylefors@gmail.com. Michel Boussinesq, Unité Mixte de Recherche 145, Institut de Recherche pour le Développement, 911 Avenue Agropolis, BP 64501, 34394 Montpellier Cedex 5, France, Tel: 33-467416162, E-mail: michel.boussinesq@ird.fr.

REFERENCES

1. Ducorps M, Gardon-Wendel N, Ranque S, Ndong W, Boussinesq M, Gardon J, Schneider D, Chippaux JP, 1995. Effets secondaires du traitement de la loase hypermicrofilarémique par l'ivermectine. *Bull Soc Pathol Exot 88:* 105–112.

2. Boussinesq M, Gardon J, Gardon-Wendel N, Kamgno J, Ngoumou P, Chippaux JP, 1998. Three probable cases of *Loa loa* encephalopathy following ivermectin treatment for onchocerciasis. *Am J Trop Med Hyg 58:* 461–469.

3. Twum-Danso NA, 2003. Serious adverse events following treatment with ivermectin for onchocerciasis control: a review of reported cases. *Filaria J 2 (Suppl 1):* S3.

4. Gardon J, Gardon-Wendel N, Demanga Ngangue, Kamgno J, Chippaux JP, Boussinesq M, 1997. Serious reactions after mass treatment of onchocerciasis with ivermectin in an area endemic for *Loa loa* infection. *Lancet 350:* 18–22.

5. Boussinesq M, Gardon J, Kamgno J, Pion SDS, Gardon-Wendel N, Chippaux JP, 2001. Relationships between the prevalence and intensity of *Loa loa* infection in the Central Province of Cameroon. *Ann Trop Med Parasitol 95:* 495–507.

6. Twum-Danso NAY, Meredith SEO, 2003. Variation in incidence of serious adverse events after onchocerciasis treatment with ivermectin in areas of Cameroon co-endemic for loiasis. *Trop Med Int Health 8:* 820–831.

7. Fobi G, Gardon J, Santiago M, Ngangue D, Gardon-Wendel N, Boussinesq M, 2000. Ocular findings after ivermectin treatment of patients with high *Loa loa* microfilaremia. *Ophthalmic Epidemiol 7:* 27–39.

8. Duke BOL, Vincelette J, Moore PJ, 1976. Microfilariae in the cerebrospinal fluid, and neurological complications, during treatment of onchocerciasis with diethylcarbamazine. *Tropenmed Parasitol 27:* 123–132.

9. Francis H, Awadzi K, Ottesen EA, 1985. The Mazzotti reaction following treatment of onchocerciasis with diethylcarbamazine: clinical severity as a function of infection intensity. *Am J Trop Med Hyg 34:* 529–536.

10. Cline BL, Hernandez JL, Mather FJ, Bartholomew R, Nunez de Maza S, Rodulfo S, Welborn CA, Eberhard ML, Convit J, 1992. Albendazole in the treatment of onchocerciasis: double-blind clinical trial in Venezuela. *Am J Trop Med Hyg 47:* 512–520.

11. Awadzi K, Dadzie KY, Schulz-Key H, Haddock DRW, Gilles HM, Aziz MA, 1985. The chemotherapy of onchocerciasis. X. An assessment of four single dose treatment regimes of MK-933 (ivermectin) in human onchocerciasis. *Ann Trop Med Parasitol 79:* 63–78.

12. Basset D, Bourée P, Basset A, Larivière M, 1989. Effets de la diéthylcarbamazine et de l'ivermectine sur la mobilisation des microfilaires d'*Onchocerca volvulus*. *Pathol Biol 37:* 668–672.

13. Richards FO Jr, Zea-Flores R, Duke BOL, 1989. Dynamics of microfilariae of *Onchocerca volvulus* over the first 72 hours after treatment with ivermectin. *Trop Med Parasitol 40:* 299–303.

14. Carme B, Boulesteix J, Boutes H, Puruehnce MF, 1991. Five cases of encephalitis during treatment of loiasis with diethylcarbamazine. *Am J Trop Med Hyg 44:* 684–690.

15. Van Bogaert L, Dubois A, Janssens PG, Radermecker J, Tverdy G, Wanson M, 1955. Encephalitis in *Loa-loa* filariasis. *J Neurol Neurosurg Psychiatry 18:* 103–119.

16. Duke BOL, Moore PJ, Vincelette J, 1975. Factors influencing the passage of *Onchocerca volvulus* microfilariae into the urine. *Tropenmed Parasitol 26:* 449–468.

17. Vuong PN, Bain O, Cabaret J, Petit G, Prod'hon J, Ranque P, Chabaud AG, 1988. Forest and savanna onchocerciasis: comparative morphometric histopathology of skin lesions. *Trop Med Parasitol 39:* 105–110.

18. Duke BOL, Wijers DJ, 1958. Studies on loiasis in monkeys. I. The relationship between human and simian *Loa* in the rain-forest zone of the British Cameroons. *Ann Trop Med Parasitol 52:* 158–175.

19. Duke BOL, 1958. Studies on the biting habits of *Chrysops*. V. The biting cycles and infection rates of *Chrysops silacea, C. dimidiata, C. langi* and *C. centurionis* at canopy level in the rain-forest at Bombe, British Cameroons. *Ann Trop Med Parasitol 52:* 24–35.

20. Duke BOL, 1964. Studies on loiasis in monkeys. IV. Experimental hybridization of the human and simian strains of *Loa*. *Ann Trop Med Parasitol 58:* 390–408.

21. Duke BOL, 2004. Failed attempts at experimental transplantation and transmission of nocturnally-periodic simian *Loa* from monkey to man. *Filaria J 3:* 5.

22. Fain A, Elsen P, Wéry M, Maertens K, 1974. Les filarioses humaines au Mayumbe et dans les régions limitrophes (République du Zaïre). Evaluation de la densité microfilarienne. *Ann Soc Belg Med Trop 54:* 5–34.

23. Kamgno J, Boussinesq M, 2001. Hyperendemicité de la loase dans la plaine Tikar, région de savane arbustive du Cameroun. *Bull Soc Pathol Exot 94:* 342–346.

24. Hawking F, Moore P, Gammage K, Worms M, 1967. Periodicity of microfilariae. XII. The effect of variations in host body temperature on the cycle of *Loa loa, Monnigofilaria setariosa, Dirofilaria immitis* and other filariae. *Trans R Soc Trop Med Hyg 61:* 674–683.

25. Nelson W, Tong YL, Lee JK, Halberg F, 1979. Methods for Cosinor rhythmometry. *Chronobiologia 6:* 305–323.

26. Hawking F, 1967. The 24-hour periodicity of microfilariae: biological mechanisms responsible for its production and control. *Proc R Soc Lond B Biol Sci 169:* 59–76.

27. Pichon G, Treuil JP, 2004. Genetic determinism of parasitic circadian periodicity and subperiodicity in human lymphatic filariasis. *CR Biol 327:* 1087–1094.

28. Hawking F, 1955. Periodicity of microfilariae of *Loa loa. Trans R Soc Trop Med Hyg 49:* 132–142.

29. Kershaw WE, 1950. Studies on the epidemiology of filariasis in West Africa, with special reference to the British Cameroon and the Niger Delta. I. Methods of survey for infections with *Loa loa* and *Acanthocheilonema perstans. Ann Trop Med Parasitol 44:* 361–378.

30. Ogunba EO, 1977. *Loa-loa* microfilaremia in the Nigerian population. *Trop Geogr Med 29:* 51–55.

31. Carme B, 1983. Etude des variations de la microfilarémie dans la filariose à *Loa loa. Ann Soc Belg Med Trop 63:* 333–339.

32. Sasa M, Tanaka H, 1972. Studies on the methods for statistical analyses of the microfilarial periodicity survey data. *Southeast Asian J Trop Med Public Health 3:* 518–536.

33. Pichon G, Thirel R, Chebret M, 1979. Nouvelle approche de la périodicité chez la filaire *Wuchereria bancrofti* var. *pacifica. Cah ORSTOM Sér Ent Med Parasitol 17:* 89–105.

34. Simonsen PE, Niemann L, Meyrowitsch DW, 1997. *Wuchereria bancrofti* in Tanzania: microfilarial periodicity and effect of blood sampling time on microfilarial intensities. *Trop Med Int Health 2:* 153–158.

35. Dzodzomenyo M, Dunyo SK, Ahorlu CK, Coker WZ, Appawu MA, Pedersen EM, Simonsen PE, 1999. Bancroftian filariasis in an irrigation project community in southern Ghana. *Trop Med Int Health 4:* 13–18.

36. Abe M, Yaviong J, Taleo G, Ichimori K, 2003. Microfilarial periodicity of *Wuchereria bancrofti* in Vanuatu. *Trans R Soc Trop Med Hyg 97:* 498–500.

37. Partono F, Purnomo, 1987. Periodicity studies of *Brugia malayi* in Indonesia: recent findings and a modified classification of the parasite. *Trans R Soc Trop Med Hyg 81:* 657–662.

38. Nogami S, Marasugi E, Shimazaki K, Maeda R, Harasawa R, Nakagaki K, 2000. Quantitative analysis of microfilarial periodicity of *Dirofilaria immitis* in cats. *Vet Parasitol 92:* 227–232.

39. Aikat TK, Das M, 1977. A modified statistical method for analysis of periodicity of microfilariae. *Indian J Med Res 65:* 58–64.

40. Bogéa T, Favre TC, Rotenberg L, Silva HS, Pieri OS, 1996. Circadian pattern of cercarial emergence in *Schistosoma mansoni* (Platyhelminthes: Digenea) from isolated *Biomphalaria glabrata. Chronobiol Int 13:* 93–101.

41. Dixit V, Pati AK, Gupta AK, Prasad GBKS, 2004. Rhythmic behaviour of *W. bancrofti* microfilaraemia in human population at Raipur. *Biol Rhythm Res 35:* 355–366.

42. Haselow NJ, Akame J, Evini C, Akongo S, 2003. Programmatic and communication issues in relation to serious adverse events following ivermectin treatment in areas co-endemic for onchocerciasis and loiasis. *Filaria J 2 (Suppl 1):* S10.

43. Thylefors B, 2008. The Mectizan Donation Program (MDP). *Ann Trop Med Parasitol 102 (Suppl 1):* S39–S44.

44. Duke BOL, 2003. Overview: report of a scientific working group on serious adverse events following Mectizan® treatment of onchocerciasis in *Loa loa* endemic areas. *Filaria J 2 (Suppl 1):* S1.

45. Duke BOL, 1970. The effects of drugs on *Onchocerca volvulus*. 4. Trials of melarsonyl potassium. *Bull World Health Organ 42:* 115–127.

46. Townson S, Tagboto SK, Castro J, Lujan A, Awadzi K, Titanji VPK, 1994. Comparison of the sensitivity of different geographical races of *Onchocerca volvulus* microfilariae to ivermectin: studies *in vitro. Trans R Soc Trop Med Hyg 88:* 101–106.

47. Awadzi K, Opoku NO, Attah SK, Addy ET, Duke BOL, Nyame PK, Kshirsagar NA, 1997. The safety and efficacy of amocarzine in African onchocerciasis and the influence of ivermectin on the clinical and parasitological response to treatment. *Ann Trop Med Parasitol 91:* 281–296.

48. Kamgno J, Gardon J, Boussinesq M, 2000. Essai de prévention des encéphalopathies à *Loa loa* post-ivermectine par l'administration d'une faible dose initiale. *Med Trop 60:* 275–277.

49. Kamgno J, Pion SDS, Tejiokem MC, Twum-Danso NAY, Thylefors B, Boussinesq M, 2007. Randomized, controlled, double-blind trial with ivermectin on *Loa loa* microfilaraemia: efficacy of a low dose (~25 µg/kg) versus current standard dose (150 µg/kg). *Trans R Soc Trop Med Hyg 101:* 777–785.

50. Chippaux JP, Ernould JC, Gardon J, Gardon-Wendel N, Chandre F, Barberi N, 1992. Ivermectin treatment of loiasis. *Trans R Soc Trop Med Hyg 86:* 289.

51. Gardon J, Kamgno J, Folefack G, Gardon-Wendel N, Bouchite B, Boussinesq M, 1997. Marked decrease in *Loa loa* microfilaraemia six and twelve months after a single dose of ivermectin. *Trans R Soc Trop Med Hyg 91:* 593–594.

52. Higazi TB, Klion AD, Boussinesq M, Unnasch TR, 2004. Genetic heterogeneity in *Loa loa* parasites from southern Cameroon: a preliminary study. *Filaria J 3:* 4.