Potential Role for Flubendazole in Limiting Filariasis Transmission: Observations of Microfilarial Sensitivity

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Abstract. Flubendazole (FLBZ) is a potent and efficacious macrofilaricide after parenteral administration. Studies in animal models and one trial in patients infected with *Onchocerca volvulus* revealed that FLBZ elicits minimal effects on microfilariae (mf). Severe complications after ivermectin (IVM) treatment of patients with high *Loa loa* microfilaraemia are of great concern. We examined the potential of FLBZ to rapidly kill *L. loa* mf, the phenomenon proposed to underlie the complications. Mf of *L. loa* were exposed to FLBZ, its reduced metabolite, albendazole, or IVM in vitro. Viability of *L. loa* mf was unaffected by FLBZ (10 µM, 72 hours); similar results were obtained with mf of *Brugia malayi*. We also measured the effects of FLBZ on transmission of mf. *Aedes aegypti* were fed FLBZ-exposed *B. malayi* mf and dissected 24 hours or 14 days postfeeding to count mf that crossed the midgut and developed to infective L₃. FLBZ impaired the ability of mf to cross the midgut, regardless of duration of exposure (≥ 2 hours). FLBZ also prevented the development of mf to L₃s, irrespective of duration of exposure or concentration. FLBZ is not microfilaricidal under these conditions; however, it blocks transmission. These results support the possibility that FLBZ may be a useful macrofilaricide in loiasis regions and may limit transmission from treated individuals.

BACKGROUND

Infections with filarial nematodes of the family Onchocercidae are among the most debilitating of the neglected tropical diseases and are estimated to affect > 150 million people.^{1,2} *Wuchereria bancrofti* and *Onchocerca volvulus*, the causative agents of lymphatic filariasis (LF) and onchocerciasis, respectively, are of greatest concern because infection can result in significant debilitation and is a major hindrance to socioeconomic development in endemic regions. *Loa loa* has received less attention. Although generally asymptomatic, loiasis can be rather disconcerting, causing pruritus, Calabar swelling, and subconjunctival migration of adult worms.³ However, a main concern regarding *L. loa* is the unacceptable incidence of severe adverse events (SAEs) observed after administration of ivermectin (IVM) for the treatment of onchocerciasis in co-endemic regions; high microfilarial loads of *L. loa* (> 30,000 mf/mL) are associated with cases of IVM-induced SAEs, including encephalopathy and death.^{4,5} The death of *L. loa* microfilariae (mf) after IVM treatment is thought to be responsible for SAEs, as IVM treatment is known to rapidly decrease circulating mf.^{6,7}

Because of the limitations imposed by IVM-associated SAEs in *L. loa* patients, mass drug administration (MDA) programs have been suspended or limited in areas that are highly endemic for *L. loa*.^{7–9} This has led the World Health Organization (WHO) to propose alternative strategies that primarily focus on interrupting transmission in these areas.^{1,2} The recommended strategy involves administration of albendazole twice yearly combined with vector control^{9–11}; however, the length of time necessary for this strategy to be effective has not been defined. Filariasis control programs would be greatly

enhanced by a macrofilaricidal drug which elicited no or slow killing of mf, thereby lessening the chances of SAEs. Flubendazole (FLBZ), a benzimidazole anthelmintic approved for the treatment of infections due to gastrointestinal nematodes of livestock¹¹ and humans,^{12–14} is an appealing candidate to address these concerns.¹⁵

As a general feature, FLBZ elicits much greater effects on adults and developmental stages of filariae than mf in animal models after parenteral administration^{16–18} and in a single *O. volvulus* human trial.¹⁹ Human trials were restricted because of problems related to the route of administration; macrofilaricidal effects are only observed with parenteral administration as available formulations of the drug have very limited oral bioavailability. Injection-based drugs have limited appeal for use in field settings, and patients in the human trial experienced injection site reactions that precluded further development. Fortunately, Johnson & Johnson have undertaken preclinical development of FLBZ²⁰ to repurpose the drug by developing an oral formulation that provides high bioavailability. A highly bioavailable, orally administered macrofilaricide with limited microfilaricidal activity would be an important contribution to campaigns to eliminate filariases as public health concerns. Considering that FLBZ has limited effects on mf in many animal models, it is important to confirm these observations by directly measuring the effects of FLBZ exposure on *L. loa* mf. This is especially true in light of the fact that orally bioavailable formulations provide much higher systemic exposure than achieved with parenteral administration,^{21,22} and the acute effects of exposure to high levels of FLBZ on mf have not been reported.

Another important consideration is that it can take weeks to months for FLBZ to produce macrofilaricidal effects.^{16–19} In the meantime, circulating mf not killed by FLBZ are available to be ingested by the mosquito host, furthering the reinfection cycle. IVM treatment of infected jirds suppresses the development of *Brugia malayi* and *Brugia pahangi* mf within a

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mosquito host that has fed on treated animals,²³ suggesting that drugs which are not directly microfilaricidal may have effects on mf that are only detectable in a model that requires development. Therefore, we conducted studies to determine if FLBZ exposure in culture elicited direct microfilaricidal activity and affected the capacity of mf to develop in a mosquito host.

$$\frac{(\# \text{ mf scoring } 3 \times 1) + (\# \text{ mf scoring } 2 \times 0.66) + (\# \text{ mf scoring } 1 \times 0.33) + (\# \text{ mf scoring } 0 \times 0)}{\text{total \# of mf}}$$

METHODS

Ethics statement. *Loa loa*. Microfilaremic blood was acquired from experimentally infected baboons²⁴ using animal procedures in accordance with animal care and use protocols at the National Institutes of Health (USA). Ethical approval for the use of baboons in this study was obtained from the Ministry of Scientific Research and Innovation of Cameroon. The use of nonhuman primates for research was approved by the Committee on the Ethical Use of Animals in Research within the Research Foundation for Tropical Diseases and Environment (REFOTDE), Cameroon.

Brugia malayi. Microfilariae and mosquitoes were supplied by the Filariasis Research Reagent Repository Center at the University of Georgia, Athens, GA. All animal protocols were reviewed and approved by the University of Georgia Institutional Animal Care and Use Committee (Approval No. A2013 11-009) and complied with U.S. Department of Agriculture regulations (USDA Assurance No. A3437-01).

Parasites. *Loa loa*. Mf were obtained from experimentally infected baboons (*Mandrillus* sp.). Blood samples were collected in EDTA-coated tubes and transported to REFOTDE at the University of Buea on ice. Blood constituents were separated on a Percoll gradient (40%, 50%, 65%) centrifuged at 4,000 $g \times 10$ minutes. Mf are found in the top layers containing platelets and lymphocytes. The top layers were removed, passed through a 5- μ m syringe filter to remove cells, and placed in a Petri dish filled with Dulbecco's modified Eagle's medium (DMEM; Wisent, St-Bruno, QC, Canada) supplemented with penicillin (100 U/mL) and streptomycin (100 μ g/mL; Invitrogen) to allow mf to migrate into the media. Mf were then concentrated by centrifuging at 2,000 $g \times 10$ minutes.

Brugia malayi. Mf were isolated via lavage from the peritoneal cavity of jirds (*Meriones unguiculatus*) > 120 days postinfection as described.^{25,26} Mf for infectivity assays were isolated from peritoneal washes via passage through a 5- μ m membrane filter and overnight incubation in DMEM medium supplemented with 100 U/mL penicillin and 100 μ g/mL streptomycin (Sigma-Aldrich, Oakville, ON, Canada) to allow mf to migrate into the media.

Culture. Mf were distributed among various treatment groups; FLBZ (10 μ M, 1 μ M, 100 nM; Epichem Pty Ltd, Bentley, WA, Australia), reduced FLBZ (10 μ M, 1 μ M, 100 nM; Epichem Pty Ltd), albendazole (10 μ M, 1 μ M, 100 nM; Sigma-Aldrich), IVM (1 μ M; Sigma-Aldrich), or 5% DMSO as a positive control for mf immobilization. All drugs were prepared in 100% DMSO and diluted in media with a final concentration of 0.1% DMSO. Incubations in drug-containing media were conducted for a maximum of 72 hours.

For infectivity assays, mf were exposed to FLBZ (10 μ M, 1 μ M, 100 nM), FLBZ-R (1 μ M) or IVM (1 μ M) for 2, 6, or 24 hours before washing in serum-free media.

Motility. Motility was scored every 24 hours using a 4-point scoring system: 3—highly active, 2—sluggish, 1—twitching of head/tail, and 0—immotile. Percent motility was determined using the following formula:

Mortality was determined as the percent of mf with motility score = 0.

Mosquito feeding. Cultured *B. malayi* mf were concentrated by centrifugation (2,000 $g \times 5$ minutes) and added to heparinized dog blood (FR3, University of Georgia) at 24–32 mf/ μ L. Mosquitoes were allowed to feed on infected blood maintained at 37°C using an artificial membrane feeding apparatus for 2 hours. Blood-fed mosquitoes were maintained in the FR3 insectary at the University of Georgia at 27°C and 80% humidity. Mosquitoes were collected from the insectary 24 hours and 14 days after feeding for either immediate dissection or fixation in 90% ethanol for later dissection.

Mosquito dissection. Freshly collected mosquitoes were kept on ice until dissection. After the removal of the wings and legs, the mosquitoes were placed on a glass slide with a drop of RPMI 1640 (Sigma-Aldrich). Midguts were carefully excised with fine forceps, taking care not to rupture the blood-engorged midgut. The midguts were coverslipped and the number of mf counted under $\times 400$ magnification.

Dissections of ethanol-fixed mosquitoes were conducted following WHO-suggested methods with a few modifications.²⁷ Mosquitoes were washed in descending dilutions of ethanol for 30 minutes (70%, 55%, 25%) followed by a final 30-minute wash in distilled water.

Midguts: The midguts were carefully excised from 24-hour fixed mosquitoes and stained whole in Mayer's haemalum for 1 hour before washing in distilled water for 15 minutes. Individual midguts were spread on glass slides and left to dry overnight before enumerating mf under $\times 600$ magnification.

L₃: Fourteen-day mosquitoes were stained in Mayer's haemalum at room temperature for 7 days. The mosquitoes were washed for 3 days in distilled water and stored in glycerol until dissection. The head, thorax, and abdomen were separated and placed into individual drops of glycerol. Fine needles were used to tease apart tissues to enumerate L₃s under a stereoscopic dissecting microscope at $\times 8$ to $\times 35$ magnification.

Statistical analysis. Statistical analyses were performed using a two-way analysis of variance in the GraphPad Prism 6 package. All statistical tests were interpreted at the 5% level of significance.

RESULTS AND DISCUSSION

Effects of flubendazole on mf of *L. loa*. Limitations of filaricidal drugs used in Africa center on the risk of IVM-induced SAEs in *L. loa* patients. Chemotherapeutic efforts would be greatly enhanced by a microfilaricide that elicited little or no damage to mf of *L. loa*. Because FLBZ is a highly effective microfilaricide, it is important to understand its effects on mf at concentrations which

FIGURE 1. Effect of flubendazole on the viability of microfilariae of *Loa loa* (B, D) and *Brugia malayi* (A, C). Motility (A, B) was scored on a 4-point scale and percent motility calculated using the formula in 5.3.4. Mortality (C, D) was calculated as the percent of microfilariae which were completely immotile and presumed dead. This figure appears in color at www.ajtmh.org.

might be obtained in plasma with an orally bioavailable formulation if it is to be used in the field.

Our results confirm the limited effect of FLBZ on mf reported in previous studies.^{16–18,28,29} Neither in utero fully stretched mf nor circulating mf exhibited detrimental effects associated with FLBZ treatment. In this study, *B. malayi* mf obtained from the jird peritoneal cavity were exposed to FLBZ. Lack of motility was considered indicative of mf death. All drug-treated groups had motility similar to that of the control (Figure 1A), with only slight motility impairment. FLBZ had no observable microfilaricidal activity (Figure 1C).

Loa loa mf were less fit under these culture conditions than *B. malayi* (Figure 1B), with the motility of mf in all treatments decreasing over time. Some differences in motility were observed between the treated groups and the control, but the

greatest difference was observed in the 5% DMSO group, which served as a positive control for motility impairment. Importantly, FLBZ had no microfilaricidal effect on *L. loa* mf. Increased mortality of drug-treated mf was only observed at the 72-hour timepoint and was < 25%. These results are promising as they suggest that FLBZ is not rapidly lethal to *L. loa* mf, which may prevent SAEs in patients with high microfilaraemia. In patients, treatment with IVM leads to a sharp decrease in mf to 40% of the original *L. loa* mf load 24 hours after treatment and to 20% of the original load by 72 hours.³⁰

Slight motility impairment was observed in drug-treated mf compared with the controls. A dramatic loss of motility was not seen, nor was there a striking microfilaricidal effect of the drugs tested (Figure 1B and D), which could have been expected with a potential SAE-inducing drug. However, this

FIGURE 2. Consequence of flubendazole (FLBZ) exposure on midgut penetration of *Brugia malayi* microfilariae to *Aedes aegypti* mosquitoes. (A) Number of microfilariae retained in the mosquito midgut 24 hours after feeding on microfilariae which were exposed to FLBZ, reduced FLBZ or ivermectin (IVM) for 24 hours in vitro. (B) Number of microfilariae retained in the mosquito midgut 24 hours after feeding on microfilariae which were exposed to FLBZ or IVM for 2, 6, or 24 hours in vitro. ***P* value ≤ 0.01 *****P* value ≤ 0.0001 . This figure appears in color at www.ajtmh.org.

FIGURE 3. Infectivity rate of *Aedes aegypti* fed on flubendazole-treated *Brugia malayi* microfilariae. Infectivity rate was calculated as the percent of mosquitoes which harbored infective L₃ larvae 14 days after feeding. This figure appears in color at www.ajtmh.org.

assay only measures direct microfilaricidal activity, and it is known that IVM, for instance, is not lethal to mf in culture.³¹ Instead, IVM-induced microfilaricidal effects require, or are greatly enhanced by, a host immune component.^{32,33} The limitation of our studies on the effect of FLBZ on *L. loa* mf in vitro lies in the absence of host components in the system; we have not demonstrated that exposure of mf in vivo to FLBZ at high concentrations may lead to effects similar to those observed with IVM or diethylcarbamazine. However, in human onchocerciasis and animal models for LF, FLBZ elicits macrofilaricidal effects over long durations.^{16,19} In addition, *O. volvulus* mf counts in skin did not diminish in human onchocerciasis patients given FLBZ parenterally until several months posttreatment. It still must be proven that an oral formulation would not provide sufficiently high exposure to produce a drastically different response on mf in vivo.

Effects of flubendazole on infectivity of mf to mosquitoes.

FLBZ requires weeks to months to produce macrofilaricidal effects.^{16–19} In the meantime, circulating mf are available to be ingested by the mosquito host, potentially furthering the infection cycle. MDA programs would benefit greatly by having a drug which is not directly microfilaricidal, and therefore potentially safe to use in *L. loa* co-endemic areas. We therefore examined the transmission-limiting ability of FLBZ by determining the infectivity of treated mf for the mosquito host, including development to the infective L₃ stage.

FLBZ exposure (24 hours) led to significantly fewer mf crossing the mosquito midgut in a concentration-dependent manner (Figure 2A). Significantly more FLBZ-exposed mf were retained in the mosquito midgut compared with the control. The 10-µM FLBZ group had the greatest impairment at all timepoints of exposure (2, 6, and 24 hours; Figure 2B). The number of mf retained in the midgut after 2- and 6-hour exposure to lower concentrations (100 nM, 1 µM) was not different from the control (Figure 2B). Interestingly, FLBZ-R (1 µM) had no effect on midgut penetration. It is important to note that this metabolite produces histological damage to adult *B. malayi*²⁸ and is known to be bioactive.³⁴

Although FLBZ exposure inhibited the ability of mf to cross the midgut, a proportion of these mf crossed into the abdominal cavity. The fate of these mf was assessed by determining if mf that had penetrated the midgut developed to the infective L₃ stage.

Infectivity in the controls was < 37.5% (Figure 3). Strikingly, exposure to FLBZ at all concentrations and for all durations

completely abolished the ability of mf to develop to infective L₃s (Figure 3).

The midgut wall of the mosquito host acts as a physical barrier to invasion by mf. Invasion into the mosquito hemocoel was once thought to require the mf to first lose their sheath before rupturing the membranes of the midgut wall using a cephalic hook.³⁵ Subsequent studies reported that mf lost their sheath as they migrate through the midgut.^{36,37}

Whether the loss of the sheath occurs before or during midgut migration, this step is an obvious requirement for development to later larval stages.

Although the factors leading to exsheathment are unknown, the process is thought to be developmentally regulated^{38,39} and can be stimulated by calcium-dependent proteases as well as other proteases or endopeptidases.^{23,40,41} Given that these factors need to be secreted from the worm to break down the sheath, it is possible that FLBZ blocks the secretion of such factors. BZs^{42,43} including FLBZ,⁴² inhibit protein secretion from gastrointestinal nematodes and *B. malayi* mf. It would not be surprising if a similar phenomenon occurs in other filarial larval stages. We did not investigate the presence or absence of sheaths on mf that penetrated the midgut. If, at lower FLBZ concentrations, mf penetrated the midgut without losing their sheath, this could account for their inability to migrate and develop to the infective stage.

CONCLUSIONS

FLBZ did not show rapid killing of *L. loa* mf in vitro. If rapid death of *L. loa* mf is responsible for the severe complications observed in IVM-treated patients, it is tempting to speculate on the safety of FLBZ in loiasis areas as it does not lead to rapid mf death. Further experiments are needed to explore the consequence of FLBZ exposure in vivo. Although not directly microfilaricidal, FLBZ completely abolished the development of *Brugia* sp. mf to infective L₃s in *Aedes aegypti*. Consequently, FLBZ treatment could block the transmission of filariae to another host. However, we do not know if mf can recover from FLBZ exposure, as is the case with adult *B. malayi*.⁴⁴

These results suggest that FLBZ could be a safe macrofilaricide to use in regions co-endemic for *O. volvulus* and *L. loa* owing to an absence of rapid microfilaricidal effects, although having the added benefit of blocking further transmission.

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REFERENCES

- Zoure H, Wanji S, Noma M, Amazigo UV, Diggle PJ, Tekle AH, Remme JH, 2011. The geographic distribution of *Loa loa* in Africa: results of large scale implementation of the Rapid Assessment Procedure for Loiasis (RAPLOA). *PLoS Negl Trop Dis* 5: e1210.
- World Health Organization, 2015. Investing to overcome the global impact of neglected tropical diseases. *Third WHO Report on Neglected Tropical Diseases*. Geneva, Switzerland: World Health Organization.
- Metzger WG, Mordmüller B, 2014. *Loa loa*—does it deserve to be neglected? *Lancet Infect Dis* 14: 353–357.
- Gardon J, Kamgno J, Folefack G, Gordon-Wendel N, Bouchite B, Boussinesq M, 1997. Marked decreases in the *Loa loa* microfilaraemia six and 12 months after a single dose of ivermectin. *Trans R Soc Trop Med Hyg* 91: 593–594.
- Boussinesq M, Gardon J, Gardon-Wendel N, Chippaux J, 2003. Clinical picture, epidemiology and outcome of *Loa*-associated serious adverse events related to mass ivermectin treatment of onchocerciasis. *Filaria J* 2: S4.
- Gardon J, Gardon-Wendel N, Demanga N, Kamgno J, Chippaux J-P, Boussinesq M, 1997. Serious reactions after mass treatment of onchocerciasis with ivermectin in an area endemic for *Loa loa* infection. *Lancet* 350: 18–22.
- Boussinesq M, Gardon J, Kamgno J, Pion S, Gardon-Wendel N, Chippaux J, 2001. Relationships between the prevalence and intensity of *Loa loa* infection in the central province of Cameroon. *Ann Trop Med Parasitol* 95: 495–507.
- Boussinesq M, Gardon J, Gardon-Wendel N, Kamgno JPN, Chippaux J, 1998. Three probable cases of *Loa loa* encephalopathy following ivermectin treatment for onchocerciasis. *Am J Trop Med Hyg* 58: 461–469.
- World Health Organization, 2012. Provisional strategy for interrupting lymphatic filariasis transmission in loiasis-endemic countries. *Report of the Meeting on Lymphatic Filariasis, Malaria and Integrated Vector Management*. Geneva, Switzerland: World Health Organization.
- World Health Organization, 2014. *Report of the Seventh Meeting of the Strategic and Technical Advisory Group for Neglected Tropical Diseases*. Available at: http://www.who.int/neglected_diseases/NTD_STAG_report_2014.pdf.
- Bradley RE, Guerrero J, Becker HN, Michael BF, Newcomb K, 1983. Flubendazole: dose range and efficacy studies against common internal parasites of swine. *Am J Vet Res* 44: 1329–1333.
- Horton RJ, 1990. Benzimidazoles in a wormy world. *Parasitol Today* 6: 106.
- Yangco BG, Klein TW, Deresinski SC, Vickery AC, Craig CP, 1981. Flubendazole and mebendazole in the treatment of trichuriasis and other helminthiasis. *Clin Ther* 4: 285–290.
- Kan SP, 1983. The anthelmintic effects of flubendazole on *Trichuris trichiura* and *Ascaris lumbricoides*. *Trans R Soc Trop Med Hyg* 77: 668–670.
- Mackenzie CD, Geary TG, 2011. Flubendazole: a candidate macrofilaricide for lymphatic filariasis and onchocerciasis field programs. *Expert Rev Anti Infect Ther* 9: 497–501.
- Zahner H, Schares G, 1993. Experimental chemotherapy of filariasis: comparative evaluation of the efficacy of filaricidal compounds in *Mastomys coucha* infected with *Litomosoides carinii*, *Acanthocheilonema viteae*, *Brugia malayi* and *B. pahangi*. *Acta Trop* 52: 221–266.
- Denham DA, Samad R, Cho SY, Suswillo RR, Skippins SC, 1979. The anthelmintic effects of flubendazole on *Brugia pahangi*. *Trans R Soc Trop Med Hyg* 73: 673–676.
- Mak JW, 1981. Antifilarial activity of mebendazole and flubendazole on *Brelinia booliati*. *Trans R Soc Trop Med Hyg* 75: 306–307.
- Dominguez-Vazquez A, Taylor HR, Greene BM, Ruvalcaba-Macias AM, Rivas-Alcala AR, Murphy RP, Beltran-Hernandez F, 1983. Comparison of flubendazole and diethylcarbamazine in treatment of onchocerciasis. *Lancet* 1: 139–143.
- Anonymous, 2014. *Johnson & Johnson Joins Public and Private Partners in the Largest Coordinated Action to Date to Eliminate or Control Neglected Tropical Diseases*. Available at: <http://www.jnj.com/news/all/johnson-and-johnson-joins-public-and-private-partners-in-the-largest-coordinated-action-to-date-to-eliminate-or-control-neglected-tropical-diseases>.
- Ceballos L, Mackenzie CD, Geary TG, Alvarez L, Lanusse C, 2014. Exploring the potential of flubendazole in filariasis control: evaluation of the systemic exposure for different pharmaceutical preparations. *PLoS Negl Trop Dis* 8: e2838.
- Longo M, Zanoncelli S, Colombo PA, Harhay MO, Scandale I, Mackenzie C, Geary T, Madril N, Mazué G, 2013. Effects of the benzimidazole anthelmintic drug flubendazole on rat embryos in vitro. *Reprod Toxicol* 36: 78–87.
- Rao UR, Kwa BH, Nayar JK, Vickery AC, 1990. *Brugia malayi* and *Brugia pahangi*: transmission blocking activity of ivermectin and brugian filarial infections in *Aedes aegypti*. *Exp Parasitol* 71: 259–266.
- Wanji S, Eyong EE, Tendongfor N, Ngwa C, Esuka E, Kengne-Ouafo A, Datchoua-Poutcheu F, Enyong P, Hopkins A, Mackenzie CD, 2015. Parasitological, hematological and biochemical characteristics of a model of hyper-microfilariaemic Loiasis (*Loa loa*) in the baboon (*Papio anubis*). *PLoS Negl Trop Dis* 9: e0004202.
- Moreno Y, Geary TG, 2008. Stage- and gender-specific proteomic analysis of *Brugia malayi* excretory-secretory products. *PLoS Negl Trop Dis* 2: e326.
- Bennuru S, Semnani R, Meng Z, Ribeiro JM, Veenstra TD, Nutman TB, 2009. *Brugia malayi* excreted/secreted proteins at the host/parasite interface: stage- and gender-specific proteomic profiling. *PLoS Negl Trop Dis* 3: e410.
- World Health Organization, 2013. *Lymphatic Filariasis: Practical Entomology. A Handbook for National Elimination Programmes*. Geneva, Switzerland: World Health Organization.
- O'Neill M, Geary JF, Agnew DW, Mackenzie CD, Geary TG, 2015. In vitro flubendazole-induced damage to vital tissues in adult females of the filarial nematode *Brugia malayi*. *Int J Parasitol Drugs Drug Resist* 5: 135–140.
- Franz M, Zahner H, Benten P, 1990. Fine-structure alterations in female *Brugia malayi* and *Litomosoides carinii* after in vivo treatment with flubendazole. *Parasitol Res* 76: 401–405.
- Ducorps M, Gardon-Wendel N, Ranque S, Ndong W, Boussinesq M, Gardon J, Schneider D, Chippaux JP, 1995. Secondary effects of the treatment of hypermicrofilaricemic loiasis using ivermectin. *Bull Soc Pathol Exot* 88: 105–112.
- Tompkins J, Stitt L, Ardelli B, 2010. *Brugia malayi*: in vitro effects of ivermectin and moxidectin on adults and microfilariae. *Exp Parasitol* 124: 394–402.
- Moreno Y, Nabhan JF, Solomon J, Mackenzie CD, Geary TG, 2010. Ivermectin disrupts the function of the excretory-secretory apparatus in microfilariae of *Brugia malayi*. *Proc Natl Acad Sci USA* 107: 20120–20125.
- McGarry H, Plant L, Taylor M, 2005. Diethylcarbamazine activity against *Brugia malayi* microfilariae is dependent on inducible nitric-oxide synthase and the cyclooxygenase pathway. *Filaria J* 4: 4.
- Urbizu L, Confalonieri A, Sánchez Bruni S, Lanusse C, Ignacio Alvarez L, 2012. Nematocidal activity of flubendazole and its reduced metabolite on a murine model of *Trichinella spiralis* infection. *Chemotherapy* 58: 295–298.
- Esslinger JH, 1962. Behavior of microfilariae of *Brugia pahangi* in *Anopheles quadrimaculatus*. *Am J Trop Med Hyg* 11: 749–758.
- Christensen BM, Sutherland DR, 1984. *Brugia pahangi*: exsheathment and midgut penetration in *Aedes aegypti*. *Trans Am Microsc Soc* 103: 423–433.

37. Agudelo-Silva F, Spielman A, 1985. Penetration of mosquito midgut wall by sheathed microfilariae. *J Invertebr Pathol* 45: 117–119.
38. Hollanda JC, Denham DA, Suswillo RR, 1982. The infectivity of microfilariae of *Brugia pahangi* of different ages to *Aedes aegypti*. *J Helminthol* 56: 155–157.
39. Griffiths K, Mayhew GF, Zink RL, Erickson SM, Fuchs JF, McDermott CM, Christensen BM, Michalski ML, 2009. Use of microarray hybridization to identify *Brugia* genes involved in mosquito infectivity. *Parasitol Res* 106: 227–235.
40. Devaney E, Howells RE, 1979. The exsheathment of *Brugia pahangi* microfilariae under controlled conditions in vitro. *Ann Trop Med Parasitol* 73: 227–233.
41. Fuhrman JA, Urioste SS, Hamill B, Spielman A, Piessens WF, 1987. Functional and antigenic maturation of *Brugia malayi* microfilariae. *Am J Trop Med Hyg* 36: 70–74.
42. Watts SDM, Rapson EB, Atkins AM, Lee DL, 1982. Inhibition of acetylcholinesterase secretion from *Nippostrongylus brasiliensis* by benzimidazole anthelmintics. *Biochem Pharmacol* 31: 3035–3040.
43. Tekwani BL, 1992. Secretory cholinesterase of *Ancylostoma ceylanicum*: effect of tubulin binding agents and benzimidazole anthelmintics. *Life Sci* 50: 747–752.
44. O'Neill M, Mansour A, DiCosty U, Geary J, Dzimianski M, McCall SD, McCall JW, Mackenzie CD, Geary TG, 2016. An in vitro/in vivo model to analyze the effects of flubendazole exposure on adult female *Brugia malayi*. *PLoS Negl Trop Dis* 10: e4698.