JOURNAL OF MULTIDISCIPLINARY & TRANSLATIONAL RESEARCH

Journal of Multidisciplinary and Translational Research (JMTR)

journal homepage: https://journals.kln.ac.lk/jmtr/

Parasitological screening of vector mosquitoes and molecular biological identification of larval filarial parasites among the wild-caught *Mansonia* mosquito species at selected areas in the district of Gampaha, Sri Lanka, a re-emerging focus of Brugian filariasis

N. Gunathilaka[1]*, U. Wimalasiri[1], N. Chandrasena[1] and R. Dalpadadu[2]

[1]*Department of Parasitology, Faculty of Medicine, University of Kelaniya, Ragama, Sri Lanka*
[2]*Regional Director of Health Services Office, Gampaha District, Gampaha, Sri Lanka*

Abstract

Brugian filariasis, a disease caused by the Protozoan parasite*Brugia malayi* has re-emerged in Sri Lanka after nearly four decades of quiescence. The *Brugia malayi* that prevailed in Sri Lanka in the past was the nocturnal periodic human strain transmitted by mosquitoes of the genus *Mansonia*. The objective of the present study was the precise identification of vector mosquitoes and parasites of the current onset of the disease. Entomological surveys were performed during September/October 2021 in Ragama Medical Officer of Health area using cattle-baited net traps. *Mansonia sp*. mosquitoes were dissected to detect the presence of larvae of the parasite. The lysate of dissected mosquitoes positive for larvae was used for the extraction of genomic DNA of the parasite, which was subjected to Polymerase Chain Reactions (PCR) aimed at molecular speciation using pan-filarial primers specific for the internal transcribed spacer region two (*ITS-2*) of the ribosomal DNA. A total of 1060 mosquitoes were tested, and that included seven mosquito species belonged to four genera. *Culex gelidus* (n=602; 56.8%) was detected as the predominant mosquito species followed by *Armigeres subalbatus* (n=420; 39.6%) *Cx. tritaeniorynchus* (n=2; 0.2%) and *Anopheles nigerrimus* (n=4; 0.4%). *Mansonia* spp. accounted for 2,7% of the total mosquito sample and among them, the presence of *Mansonia annulifera* was 1,2% of the total (n=20), *Ma. uniformis* was 0.9% (n= 10) and *Ma. Indiana* was 0.2% (n= 2). About 18.7% (n=6) of *Mansonia* mosquito collection was positive for filarial larvae. Among them, 15.6% (n=5) was *Mansonia annulifera* while (3.1%; n=1) was *Ma. uniformis.* The PCR products of all tested samples corresponded to the band size of 625 bp, specific to *B. malayi* confirming the identity of the parasite. *Mansonia annulifera* and *Ma. uniformis* were confirmed as vectors of the re-emerged *B. malayi* (nocturnally sub-periodic) in Gampaha district. The role of other mosquito vector species would require investigation by vector incrimination and xenomonitoring-based approaches.

Keywords: *Brugia malayi*, Brugian filariasis, *Mansonia*

Article info

Article history:
Received 30th April 2024
Received in revised form 12th June 2024
Accepted 20th June 2024
Available online 30th June 2024

ISSN (E-Copy): ISSN 3051-5262
ISSN (Hard copy): ISSN 3051-5602
Doi:
ORCID iD: https://orcid.org/0000-0002-2690-8565
*Corresponding author:
E-mail address: n.gunathilaka@kln.ac.lk (N. Gunathilaka)

Introduction

Lymphatic filariasis (LF) is a mosquito-borne disease placed in the category of neglected tropical disease (NTD) and is targeted by the World Health Organization (WHO) for elimination by 2030 (WHO, 2024). The causative filarial parasite species are *Wuchereria bancrofti, Brugia malayi* and *B. timori*. Although not fatal, LF causes significant acute and chronic morbidities such as acute lymphangitis, lymphangioadenitis, acute epididymo-orchitis, lymphoedema, elephantiasis and hydrocele. The estimated loss of disability-adjusted life years (DALYs) due to LF is 4.4 million and 1.3 million in men and women, respectively (Nutman, 2013). The chronic morbidities lead to physical disfigurement, permanent deformities, and disabilities resulting in physical, psychosocial, and economic problems.

The distribution of LF is mostly confined to the tropical and subtropical regions of the world with *W. bancrofti* causing 90% of the infections while *B. malayi* is responsible for most of the remaining 10% of infections (WHO, 2015). Brugian filariasis affects the Asian region with *B. malayi* in South and Southeast Asia (the predominant species in Indonesia, South India, Vietnam, Thailand and Philippines) while *B. timori* is restricted to the islands of Timor-Leste (Noordin, 2007; Ottesen et al., 1997; Simonsen et al., 2014). The situation in Sri Lanka differed as the control efforts of the National Anti-filariasis Campaign (AFC) which was launched in 1947 was successful in clearing the nocturnal periodic *B. malayi* infections in the late 1960s by the removal of the aquatic larval host plants of vector mosquito *Mansonia* sp. (Gautamadasa, 1986; Schweinfurth, 1983). Thus, cases of Brugian filariasis were not reported in the country for almost four decades.

A preliminary vector survey previously carried out in the Puttalam district, a different administrative district in Sri Lanka, reported that 33.3% (n=4) of *M. annulifera* and 50% of *M. uniformis* (n=10) had filarial larvae of *B. malayi.* It was reported further that 77% of *M. indiana* (n=14) had filarial larvae of *B. malayi*, revealing for the first time that *M. indiana* also is a potential vector of filarial parasites in Sri Lanka (Nimalarathna et al 2022).

Sri Lanka's AFC launched the national LF elimination program in 2002 based on the World Health Organization (WHO)'s Global Program for Elimination of LF (GPELF) using mass administration of preventive chemotherapy (MDA) (WHO, 2021). Five successful rounds of MDA with diethylcarbamazine citrate and albendazole were administered from 2002-2005 in the three LF endemic provinces, namely Northwestern, Western and Southern provinces, and the country received validation by WHO of having eliminated LF as a public health problem in 2016 (WHO, 2021). However, during the post-MDA and post-validation surveillance activities, sporadic occurrence of *B. malayi* cases were reported along the Northwestern, Western and Southern provincial coastal areas with some aggregation in the district of Puttalam in Northwest province and in the district of Kalutara in Southwest province (Chandrasena et al., 2016; MOH, 2021). The infecting strain exhibited nocturnal sub-periodicity and was linked to a zoonotic origin with dogs and cats being the main reservoir hosts (Mallawarachchi et al., 2021, 2018). Since mosquitoes of the genus *Mansonia* were implicated as vectors of the *B. malayi* strain that prevailed in Sri Lanka in the past (Dissanaike, 1986), this study investigated the role of *Mansonia* sp. mosquitoes in the transmission of the re-emerged zoonotic strain of *B. malayi*.

Material and Method

Selection of the study area

The administrative district of Gampaha is situated in the LF endemic Western province of Sri Lanka, covering a land area of 1287 km^2 and, is the second most populous district in the country. Several case reports of Brugian filariasis in the district with a high canine and feline reservoir of infection have indicated ongoing transmission in the region (Chandrasena et al 2016, Mallawarachchi et al 2018a, Mallawarachchi et al 2018b; Mallawarachchi et al., 2021). Based on the presence of human and animal Brugian filariasis transmission in recent years, Ragama Medical Officer of Health (MOH) area was selected for the present study.

Collection of mosquitoes and species identification

The entomological survey was conducted at selected locations in the selected MOH area during September/October 2022 using Cattle-Baited Net Trap (CBNT) collection method following the standard guidelines described by the WHO (WHO, 1992). The traps were set up at each location in evenings (17:00 – 18.00 h) and the mosquitoes were captured from 21:00 to23:00h using a battery-operated aspirator. Collected mosquitoes were put into paper cups covered with a mosquito net and transferred safely to the laboratory at the Department of Parasitology, Faculty of Medicine, University of Kelaniya, Sri Lanka. The captured mosquitoes were sacrificed with a cold shock and separated into the genus level by key morphological characters as described by Amerasinghe et al., 1995). The species-level identification was performed using standard taxonomic keys (Chelliah and Jeyasekera, 1981; Rattanarithikul et al., 2005). Species under the genus *Mansonia* were taken for parasitological and molecular screening for the presence of filarial parasites.

Mosquito dissection and microscopic screening for microfilaria

Each mosquito was placed on a clean glass slide and the head, thorax and abdomen were separated using dissection needles. Each body part was placed on a drop of normal saline and the tissue was finely teased using fine needles. The teased tissues were examined, under the x100 magnification, for the presence of filarial larval stages using a binocular compound microscope with a mounted microscope camera. The mosquitoes that had filarial larval stages L1, L2, or L3 in any of the body segments were taken as infected mosquitoes.

Molecular screening for species identification of filarial parasites

Genomic DNA extraction

The lysate of the dissected mosquitoes (only the head and thorax parts) with filarial larvae were taken for genomic DNA extraction. The extraction was carried out using 300 µl of MightyPrep reagent according to the manufacturer's instructions (Takara Bio Inc, Cat. #9182). The mixture was vortexed and incubated at 95°C for 10 minutes and, then was . centrifuged at 17000 xg for 2 minutes. The supernatant was subjected to Polymerase Chain Reaction (PCR) for the amplification of a DNA segment specific to filarial parasites as described below.

Polymerase Chain Reaction

The primer used in the PCR was the pan-filarial primer (DIDR-F1 = 5' -AGT GCG AAT TGC AGA CGC ATT GAG -3' and DIDR-R1 = 5' -AGC GGG TAA TCA CGA CTG AGT TGA -3') designed by Rishniw et al (Rishniw *et al.,* 2006). This is a species-specific primer that amplifies the internal transcriber spacer region-2 (ITS-2) of six different filarial species (*Dirofilaria immitis*, *D. repens*, *Brugia malayi*, *B. pahangi*, *Acanthocheilonema reconditum* and *A. dracunculoides*). The HotStarTaq Plus Master Mix kit (Qiagen, Germany) was used in the amplification reaction according to the manufacturer's guidelines. The reaction mixture consisted of 12.5 µL of 2x HotStarTaq Plus Master Mix, 2.5 µL of 10x Coral Load concentrate buffer, 1.25 µL of each primer, and 1.0 µL of the DNA extract. The total volume was brought up to 20.0 µL by adding nuclease-free water. DNA of *D. repens* and *B. malayi* were used as positive controls while distilled water was used as the negative control.The PCR protocol that followed using a LifeECO Thermal Cycler (Bioer, China) consisted of an initial denaturing step at 95^0 C for 5 min and 30 cycles of denaturing (30s at 94 ^{0}C), annealing (30s at 58.0 ^{0}C), extension (40s at 72 ^{0}C), a final extension (7 min at 72 ^{0}C), and cooling at 4 ^{0}C.

Identification of the PCR product

A 1.5 % agarose gel with ethidium bromide (0.5 µg/ml) was prepared using agarose powder (Agarose LE) with 1 x TAE buffer. 4 µL of the PCR reaction was used for gel electrophoresis that was carried out at 100 V for 30 minutes. . The migrated DNA in the gel was visualized and captured under UV illumination using a UV transilluminator (Maestrogen, Japan) and was compared with the marker and the specific band sizes of each species.

Results

Species composition and parasitological screening of *Mansonia* mosquitoes

The total of 1060 mosquitoes that were tested included seven mosquito species belonging to four genera. *Culex gelidus* (n=602; 56.8%) was detected as the predominant mosquito species followed by *Armigeres subalbatus* (n=420; 39.6%) *Cx. tritaeniorynchus* (n=2; 0.2%) and *Anopheles nigerrimus* (n=4; 0.4%). *Mansonia* spp. accounted for 2,7% of the total mosquito sample and among them, the presence of *Mansonia annulifera* was 1,2% of the total (n=20), *Ma. uniformis* was 0.9% (n= 10) and *Ma. Indiana* was 0.2% (n= 2). 18.7% (n=6) of *Mansonia* mosquito collection was positive for filarial larvae. Among them, 15.6% (n=5) was *Mansonia annulifera* while (3.1%; n=1) was *Ma. uniformis*. The presence of a larval stage of filarial worm identified from a dissected mosquito is illustrated in Figure 1.

Figure 1: Presence of the larval stage of a filarial parasite in the dissected thorax region of a Mansonia mosquito under 100x magnification.

Molecular biological characterization of filarial parasites

Extracted DNA from all the infected *Mansonia* mosquitoes elicited the expected band of 615 bp DNA, confirming the filarial larvae as that of *B. malayi*. The photograph of the agarose gel electrophoresis is given in Figure 2.

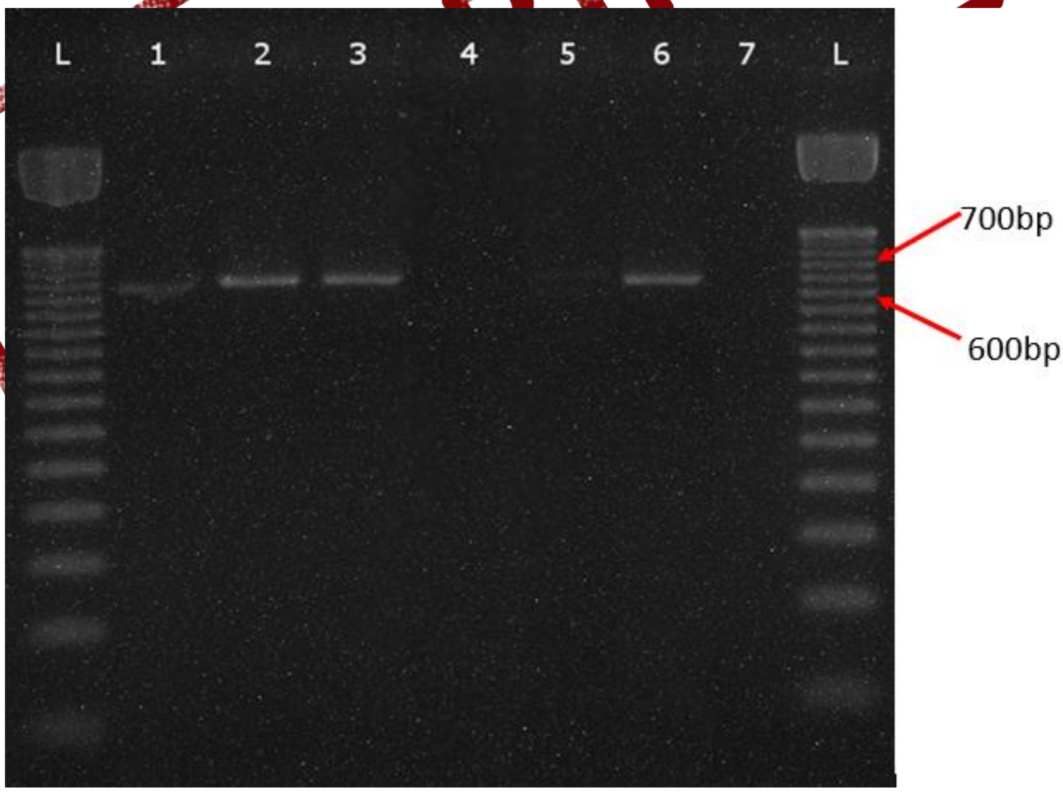

Figure 2: The results of gel electrophoresis targeting the internal transcriber spacer region-2 (ITS-2) of filarial parasites in *Mansonia* mosquitoes. **Lane L;** 50 bp ladder, **Lane 1 & 2;** *Ma. annulifera* positive for *B. malayi, Lane 3; M. uniformis* positive for *B. malayi*, *Lane 4; M. Indiana* negative for *B. malayi* , **Lane 5;** *M. uniformis* negative for *B. malayi* , **Lane 6;** Positive control and **Lane 7;** Negative control.

Discussion

The re-emerged *B. malayi* strain in Sri Lanka was reported as a novel genetic variant with a higher sequence homology to *B. pahangi* than *B. malayi* (Mallawarachchi et al., 2021). Hence an important aspect was to determine the transmission dynamics of this variant strain for epidemiological aspects and purposes of control. By combining both traditional entomology and novel molecular methods, the current study established that *M. annulifera*, and *M. uniformis* were the vectors of the re-emerged *B. malayi* strain currently circulating among humans and zoonotic reservoir hosts in the administrative district of Gampaha in Sri Lanka.

As reported by Edeson and Wilson (1964), mosquitoes of the genera *Mansonia*, *Anopheles* and *Aedes* were the principal mosquito vectors of *B. malayi* in Sri Lanka. The nocturnally periodic form of *B. malayi* was transmitted by *Mansonia* and some *Anopheles* mosquitoes breeding in open swamps and rice growing areas. These mosquitoes are night-biting, and the parasite strain seemed to affect only humans as natural animal infections were rare and experimental infections were not retained (John and Petri, 2006). The nocturnally sub-periodic form was reported as transmitted by *Mansonia* mosquitoes in forest swamps where mosquitoes may bite at any time in the shade (Edeson and Wilson, 1964). In this form of Brugian filariasis, natural zoonotic infections were common and cats, dogs, slow lorises, acquire the infection from humans and also serve as reservoir hosts.

Brugia pahangi is an animal filaria parasite mainly affecting domestic cats and other animals in Malaysia, Thailand and Indonesia (Mak et al., 1980) and the mosquito *Armigeres subalbatus* has been incriminated as the vector in suburban Kuala Lumpur in Peninsular Malaysia (Muslim et al., 2013) .The close genetic relation of the re-emerged *B. malayi* strain to *B. pahangi,* which has been recently reported among canines in Sri Lanka (Rathnayake et al., 2022), suggested the possibility of a wider range of mosquito vectors involved in transmission of this variant of *B. malayi*.

Treating human infections and implementing conventional measures that were utilized for clearing the nocturnally periodic strain of *B. malayi*, namely the removal of aquatic host plants, may not suffice in clearing the variant sub-periodic strain of *B. malayi* mainly because the disease has re-emerged. Therefore, further entomological studies are recommended using both outdoor and indoor mosquito collections from different geographical regions to identify mosquito vectors involved in both animals to human and animal to animal transmission.

Conclusion

The mosquito species, *M. annulifera* and *M. uniformis* were implicated as vectors of the variant *B. malayi* strain that has re-emerged in Sri Lanka. The re-emergence of *B, malayi* may jeopardize the national LF elimination efforts. Further entomological surveys and a one health approach is recommended to prevent the re-establishment of Brugian filariasis in the country.

Funding

Molecular work of the research component was carried out from the consumables purchased from the National Research Council, Sri Lanka (Grant No. NRC 16-142).

Acknowledgement

We would like to acknowledge the Entomology field staff attached to the Entomology Office of the Regional Director of Health Services, Gampaha District Sri Lanka for conducting field surveys and providing samples.

Conflict of interest

The authors declare that they have no conflict of interest.

References

Amerasinghe, F.P. (1995). Illustrated keys to the genera of mosquitoes (Diptera: Culicidae) in Sri Lanka. Journal of National Science Council of Sri Lanka, 23(4),183–211.

Anti-Filariasis Campaign. Annual Statistical Bulletin (2015) Anti-Filariasis Campaign, Ministry of Health and Indigenous Medicine Sri Lanka.

Anti-Filariasis Campaign. Annual Statistical Bulletin (2019-2021) Anti-Filariasis Campaign, Ministry of Health and Indigenous Medicine Sri Lanka.

Chandrasena, T.G.A.N., Premaratna, R., Samarasekera. D.S., & de Silva N.R. (2016). Surveillance for transmission of lymphatic filariasis in Colombo and Gampaha districts of Sri Lanka following mass drug administration. Transactions of the Royal Society of Tropical Medicine and Hygiene, 110(10), 620-622.

Chelliah, R.V. (1984). Keys and illustrations to the genera of mosquitoes of Sri Lanka (Diptera: Culicidae). Contributions of the American Entomological Institute, 7(4), 1–84.

Dissanaike, A.S. (1986). A review of Brugia species with special reference to B. malayi and zoonotic infection. Tropical Biomedicine, 3, 67–72.

Edeson, J.F.B., & Wilson, T. (1964). The Epidemiology of Filariasis due to Wuchereria bancrofti and Brugia malayi. Annual Review of Entomology, 9(1), 245–268.

Gautamadasa, C.H. (1986). A historical review of Brugian filariasis and its present status in Sri Lanka. (Doctor of Medicine dissertation, University of Colombo, Sri Lanka).

John, David T, Petri, William A. (2006), Markell and Voge's Medical Parasitology (9th ed.), St. Louis: Saunders Elsevier.

Mak, J.W., Yen, P.K., Lim, K.C., & Ramiah, N. (1980). Zoonotic implications of cats and dogs in filarial transmission in Peninsular Malaysia. Tropical and Geographical Medicine, 32, 259–264.

Mallawarachchi, C.H., Chandrasena, T.G.A.N., Premaratna, R., Mallawarachchi, S.M.N.S.M., & de Silva NR. (2018a). Human infection with sub-periodic Brugia Spp. in Gampaha District, Sri Lanka; a threat to filariasis elimination status? Parasite and Vectors, 11(1),68.

Mallawarachchi, C.H., Chandrasena, N.T.G.A., Wickremesinghe, S., Premaratna, R., Mallawarachchi, N.S.M.S.M., Gunawardane, N.Y.I.S., & de Silva NR. (2018b). A preliminary survey of filarial parasites in dogs and cats in Sri Lanka. PLoS One, 13(1-11), e0206633.

Mallawarachchi, C.H., Chandrasena, T.G.A.N., Withanage, G.P., Premaratna, R., Mallawarachchi, S.M.N.S.M., Gunawardane, N.Y., Dasanayake, R., & de Silva, N.R. (2021). Molecular Characterization of a Reemergent Brugia malayi Parasite in Sri Lanka Suggestive of a Novel Strain. Biomed Research International, 2021, 9926101.

Muslim, A., Fong, M.Y., Mahmud, R., Lau, Y.L., & Sivanandam, S. (2013). Armigeres subalbatus incriminated as a vector of zoonotic Brugia pahangi filariasis in suburban Kuala Lumpur, Peninsular Malaysia. Parasite and Vectors, 6, 219.

Nimalrathna, S., Mallwarachchi, C.T.G.A.N., Chandrasena, T.G.A.N., De Silva, N., Kimber, M., De Silva, N.R., & Harischandra, H. (2022). A preliminary survey for filarial parasites among dogs and cats in Mahawewa, Puttalam and their vector identification. International Journal of Infectious Diseases, 116,122-123.

Noordin, R. (2007). Lymphatic Filariasis and the Global Elimination Program. The Malaysian Journal of Medical Sciences, 14(1), 1–3.

Nutman, T.B. (2013). Insights into the Pathogenesis of Disease in Human Lymphatic Filariasis. Lymphatic Research and Biology, 11(3), 144–148.

Ottesen, E.A., Duke, B.O.L., Karam, M., & Behbehani, K. (1997). Strategies and tools for the control/elimination of lymphatic filariasis. Bulletin of the World Health Organization, 75(6), 491–503.

Rathnayake, S., Chandrasena, N., Wijerathna, T., Mallawarachchi, H., & Gunathilaka, N. (2022). Canine filarial species in selected lymphatic filariasis endemic and non-endemic areas in Sri Lanka. Parasitology Research, 121(7), 2187-2191.

Rattanarithikul, R., Harrison, B.A., Panthusiri, P., & Coleman, R.E. (2005). Illustrated keys to the mosquitoes of Thailand 1, background; geographic distribution; list of genera, subgenera and species; and a key to the genera. The Southeast Asian Journal of Tropical Medicine and Public Health, 36(1), 1–80.

Rishniw, M., Barr, S.C., Simpson, K.W., Frongillo, M.F., Franz, M., & Alpizar, J.L.D. (2006). Discrimination between six species of canine microfilariae by a single polymerase chain reaction, Veterinary Parasitology, 135(3-4), 303-314.

Schweinfurth, U. (1983). Filarial diseases in Ceylon: a geographic and historical analysis. Ecology of Disease, 2(4), 309–319.

Simonsen, P. E., Fischer, P.U., Hoerauf, A., & Weil, G.J. (2014). The Filariases in Manson's Tropical Diseases (Twenty-third edition). Elsevier Saunders, 10(54), 737-765.

World Health Organization (2000). Preparing and implementing a national plan to eliminate lymphatic filariasis (in countries where onchocerciasis is not co-endemic) World Health Organization, Geneva, WHO/CDS/CPP/CEE 200016.

World Health Organization (16 April 2023). Maldives and Sri Lanka eliminate lymphatic filariasis. Retrieved from http://wwwsearo.who.int/mediacentre/releases/2016/1626/en/

World Health Organization (Ed.). (2015). Investing to overcome the global impact of neglected tropical diseases: Third WHO report on neglected tropical diseases. Geneva: World Health Organization

World Health Organization (1992), Entomological Field Techniques for Malaria Control Part 1 Learner's Guide, World Health Organization (WHO), Geneva, Switzerland, 1992.

World Health Organization: NTD Roadmap 2021-2030. Proposed goals and milestones for each NTD 2019.