Maintenance of the human filarial parasitic nematode *Brugia malayi* life cycle in the Mongolian gerbil (*Meriones unguiculatus*)

ANDREW STEVEN, SHREE HEGDE, JESSICA DAGLEY, BETHANY BATTERLEY, MARK TAYLOR and JOSEPH TURNER

Centres for Neglected Tropical Diseases and Drugs and Diagnostics, Liverpool School of Tropical Medicine, Liverpool

Correspondence: Andrew.Steven@lstmed.ac.uk

Abstract

Brugia malayi is a human filarial parasitic nematode (roundworm) and a cause of lymphatic filariasis (LF) or elephantiasis, a condition characterised by swelling of the genitals and limbs. LF affects over 51 million people in 72 countries throughout the tropics and sub-tropics of Asia, Africa, the Western Pacific, parts of the Caribbean and South America. To combat this disease, teams at the Liverpool School of Tropical Medicine (LSTM) have discovered a symbiotic bacterium called *Wolbachia* which the worms have become dependent upon for their growth, development and survival. *Wolbachia* is therefore an excellent selective drug target and the use of antibiotics to eliminate *Wolbachia* leads to the death of the parasite. The screening of drug libraries to discover new anti-Wolbachia antibiotics requires the laboratory maintenance of the *Brugia malayi* (*B. malayi)* life cycle in Mongolian gerbils (*Meriones unguiculatus*) and *Aedes aegypti* mosquitoes. Here we review some advances in the life cycle maintenance that address the 3Rs (Replacement, Reduction, Refinement) to improve Animal Welfare and summarise some of the biological advances we have achieved using this model system.

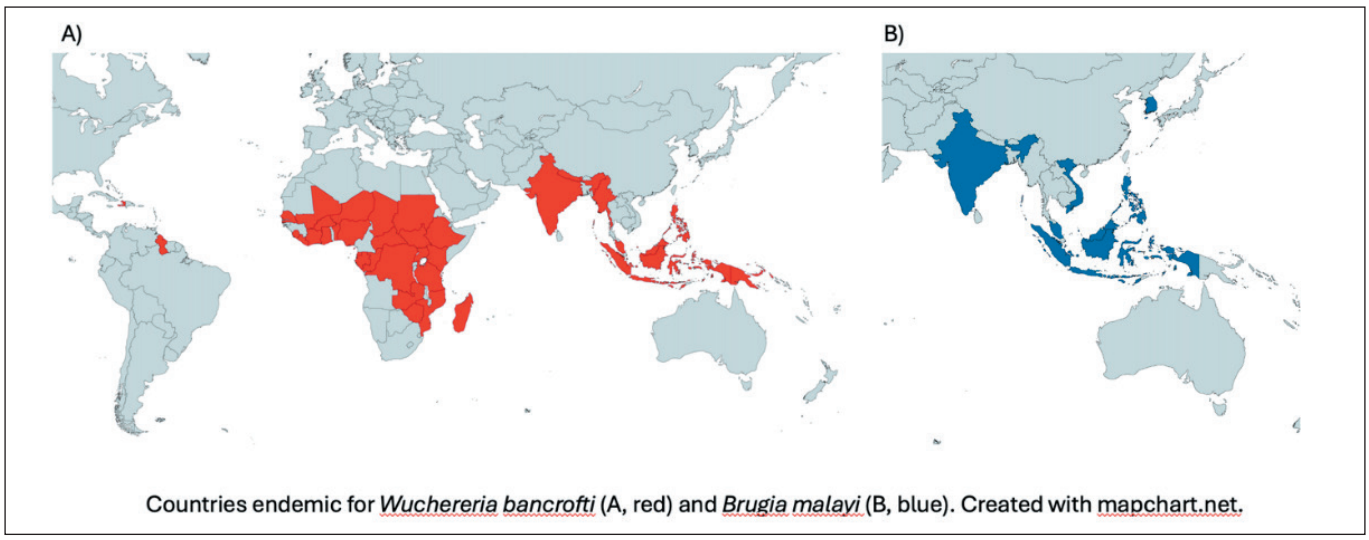

Figure 1. Global distribution of the causative agents of lymphatic filariasis (LF).
Wuchereria bancrofti (red) cause 90% of LF infections and is ubiquitous in equatorial climates. *Brugia malayi* (blue) make up the remaining LF cases in India and South-East Asia.

Introduction

Brugia malayi is a parasitic filarial nematode that causes lymphatic filariasis (LF), which is a debilitating tropical disease primarily affecting the lymphatic system. It is one of the three filarial species, alongside *Wuchereria bancrofti* and *Brugia timori* responsible for this condition.[1,2] *B. malayi* is transmitted to humans through the bite of infected mosquitoes, particularly those of the *Mansonia* and in some foci *Aedes* and *Anopheles* genera. Once inside the human host, *B. malayi* matures and reproduces in the lymphatic vessels, leading to inflammation, lymphoedema and in chronic cases, elephantiasis.[2]

Endemic primarily in Southeast Asia and parts of the Indian subcontinent, *B. malayi* represents a significant public health concern in these regions (Figure 1). Control efforts, including mass drug administration (MDA) and vector control have been implemented as part of global initiatives led by the World Health Organisation (WHO) to eliminate LF as a public health problem.[2,3] The elimination of *B. malayi* is complicated by reservoirs of infection in domestic cats and dogs in Southeast Asia.

To maintain the life cycle of *B. malayi* in a controlled environment, the mosquito *Aedes aegypti* is reared within a temperature and humidity-controlled insectary.

Mosquito larvae go through multiple growth stages known as instars. They have three to four instars, during which they moult several times. Each instar lasts about a day or two and the larvae grow larger. So larval stage 1 (L1) and larval stage 2 (L2). This carries on until larvae stage 4 (L4) when it turns into a pupa before emerging as an adult. Adult female mosquitoes are infected with the L1 parasites which moult and develop into L2 larvae in the flight muscles before moulting again to become infective L3 larvae (Figure 2). L3 produced by these infected mosquitoes are subsequently injected into Mongolian gerbils.[4]

Mosquitoes bite a mammalian host and ingest an infectious blood meal containing *B. malayi* microfilaria (mf). This parasitic worm undergoes several developmental stages within the intermediate vector host to become infective L3 larvae. L3 enter the human host through the wound made by the blood feeding mosquito, migrate to the lymphatics and moult and develop into fourth-stage larvae before the final moult to become male and female adult parasites, which mate and produce mf, which migrate into the peripheral blood for transmission to mosquito vectors. In laboratory infections, if infected via the intraperitoneal route, all mammalian life cycle stages (L3, L4, L5 pre-adults, adult parasites and mf) are confined to the peritoneal cavity, which facilitates their collection in timed infections.

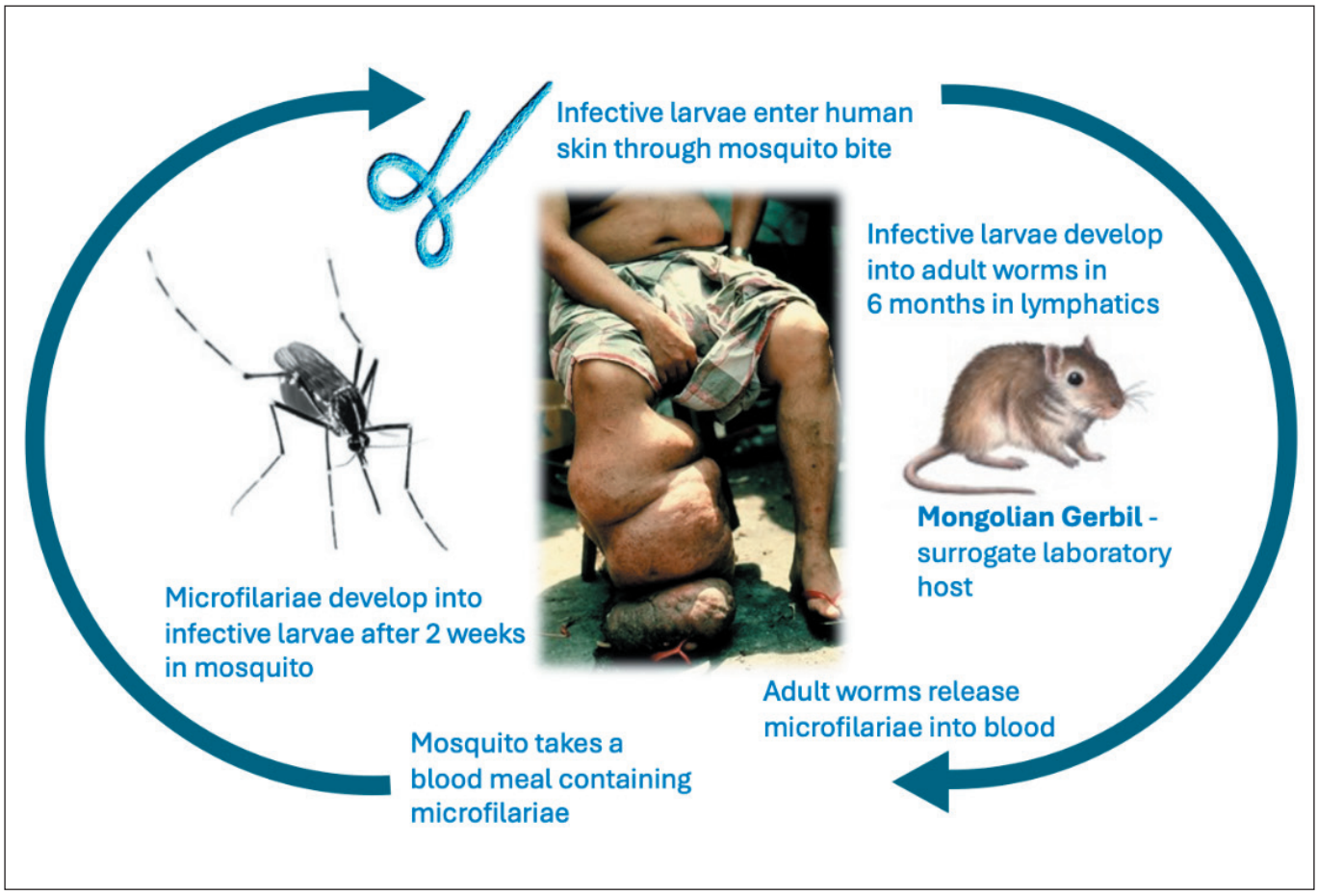

Figure 2. Life cycle of *Brugia malayi*.

Materials and methods

The maintenance of the mosquito life cycle is a 4-week process starting with the setting up of four filter papers containing approximately 2000 plus eggs from the female *Aedes aegypti* (black eye susceptible strain), (Figure 3). This particular strain was founded in the 1930s in Liverpool, United Kingdom (UK), originally showing 30% filaria-susceptible and 70% non-susceptible with subsequent crosses producing the highly susceptible black-eye strain.[5,6,7,8] Eggs that have been laid on cotton wool pads by female mosquitoes are placed into a plastic tray (L. 300mm x W. 210mm x H. 80mm) with 250mls of distilled water with a 600mg yeast tablet. Eggs are left to emerge for two days and the L1 larvae are split into another 12 to 15 trays at a density of approximately 250 larvae per tray. After a further five days the larvae progress from the L1 to L4 stage and will then develop into pupae (Figure 3). These are collected by combining three trays of pupae into a 100 x 60mm net and pupae placed into a 250ml polystyrene pot containing 100ml of distilled water. Each subsequent pot collected is placed into an insect cage measuring (300 x 300 x 300mm) (Bugdorm-1)[9] allowing them to emerge as adult mosquitoes. They are maintained with 10% sugar solution on a cotton wool pad which is replenished daily.

The *Aedes aegypti* mosquito undergoes four larval stages before becoming a susceptible insect vector. Blood fed female *Aedes aegypti* mosquitoes (1) lay eggs on cotton wool pads (2), which are then floated out to hatch as and turn into larvae stages L1-L4 (3). These then turn into pupae (4). The life cycle is described above.

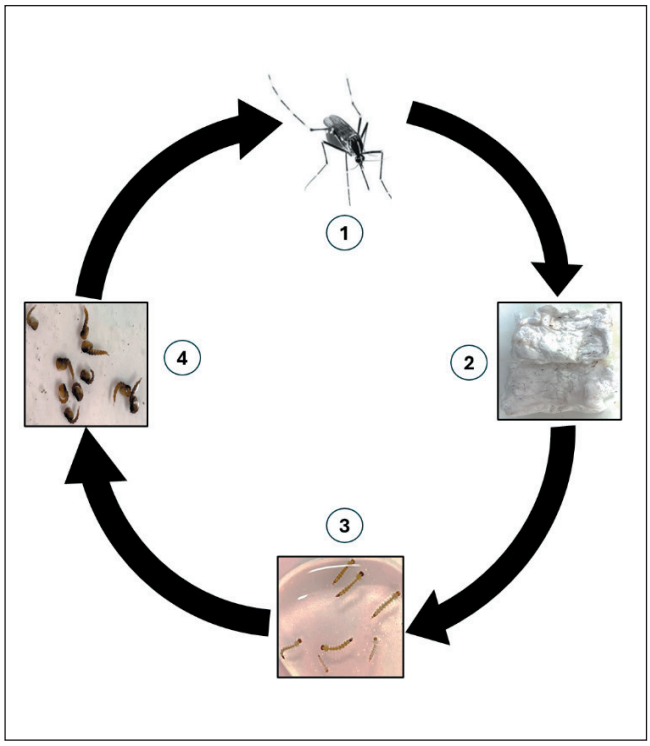

Figure 3. *Aedes aegypti* life cycle.

Animals

Mongolian gerbils (*Meriones unguiculatus*) are used by Liverpool School of Tropical Medicine (LSTM) researchers and housed in the Biomedical Service Unit (BSU) at the University of Liverpool (UoL). *B. malayi* life cycle maintenance is carried out in Rj:MON gerbil strains[10] with male gerbils used due to their higher susceptibility to infection.[11] The gerbils are outbred from Janvier labs, Europe.[12] All gerbils are housed and maintained under specific pathogen-free (SPF) conditions at the BSU in high efficiency particulate air (HEPA) filtrated individually ventilated caging (IVC). Animals are housed at a temperature range of 20 to 23°C with a relative humidity of 50% +/- 10%, an artificial 12-hour light-dark cycle and 15 air changes per hour. An acclimatisation period of 7 days is required before the start of any procedure. Gerbils are placed on 5R53 – PicoLab® Rodent Diet 20 EXT with access to an automated watering system. Animals are housed in Tecniplast GR1800 Green Line IVCs[13] with Lignocell select bedding and a metal u-shaped tunnel for enrichment. Male gerbils aged between 4-6 month-old weighing 80 to 100g are used at the start of all procedures.

Aedes aegypti mosquitoes are bred in house from fresh egg papers laid on clean damp cotton wool pads (Figure 3 (2)) in a plastic dish. Once the mosquitoes have hatched, they are reared within a temperature and humidity-controlled insectary at 26 to 28°C / 86 to 88% with an artificial 12-hour light-dark cycle. Mosquitoes are maintained on a 10% sugar/water (sucrose) solution using damp cotton wool pads which are placed on the top of the cage and changed daily.

Gerbil lavage

Abdominal lavages are carried out on male gerbils (6 to 18 months post infection) to harvest mf using the method of Griffiths *et al.*[14] Gerbils are anaethetised with isoflurane at a concentration of 2L/minute and 1.5L/minute of pure oxygen for five minutes. Once anaethetised, animals are placed under a sterile CAT 2 safety hood before the administration of the catheter 20G x 32mm (Troge) into the abdominal cavity. During the procedure the gerbils are fully and continuously anaesthetised whilst in the hood so they do not wake up (Figure 4 A,B). 5mls of warm modified RPMI 1640 media containing 1% penicillin-streptomycin is injected into the abdominal cavity of the gerbil (Figure 4C) and the needle is removed leaving the catheter in place. A 30ml sterile Universal collection tube is placed under the catheter and the abdominal region of the gerbil is massaged where it will release the mf. The mf are continuously collected until a total of approximately 4mls is retrieved (Figure 4D). It takes approximately three minutes to collect the 4mls from the gerbil as one sample and this is all done in one subtraction. Once the procedure is finished the gerbil is administered oxygen until recovered then returned to the

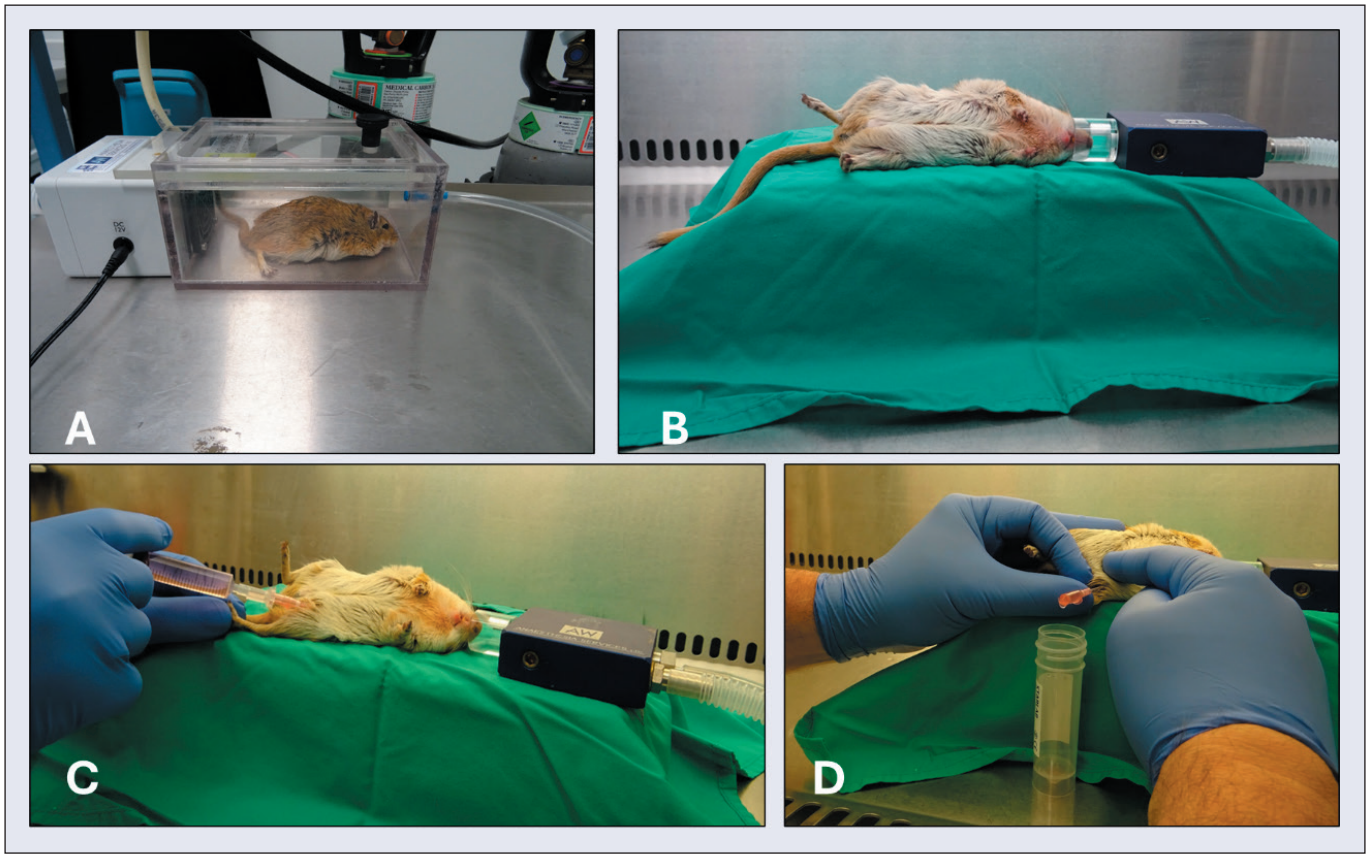

Figure 4. Procedure for gerbil lavage.

cage. Gerbil lavage is a refinement because it allows for valuable biological data collection using less invasive, lower-stress and more humane techniques, improving the welfare of the animals while reducing overall numbers required for research. The gerbils experience shorter procedure times and faster recovery.

The procedure and how it was carried out outlined under gerbil lavage on this paper. This procedure is performed on the gerbil once per month as the gerbil must be left for a month before it can be abdominally lavaged once again. This is outlined in our Home Office licence.

Preparing mf for mosquito infection

Once the abdominal lavage of the gerbil has been applied, the mf is purified using a PD10 column before feeding to mosquitoes. The column is purged with 50ml of modified RPMI media whereupon mf is introduced and purified mf separated from the gerbil host cells are collected into 15ml falcon tubes. The mf is subsequently counted by microscopy and can generate a concentration of approximately 400,000–1,500,000 mf per gerbil (Figure 5).

Mf (arrows) are observed under microscope at a x10 magnification after being diluted from a higher concentration. The size of the mf range between 177 to 230µm in length and 5 to 7µm in width.

Adult female mosquitoes can be fed an infected blood meal after two weeks and are allowed to feed for up to two hours using an artificial feeding system (Hemotek®) at a concentration of 15 to 20,000 mf/ml in human blood (Blood bank, Liverpool UK), The Hemotek® is heated to 37°C and a feeding disc containing the infected blood meal, covered by a parafilm® membrane (Figure 6A), is placed on top of the cage which the mosquitoes feed upon (Figure 6 B,C). Once the feeding is complete,

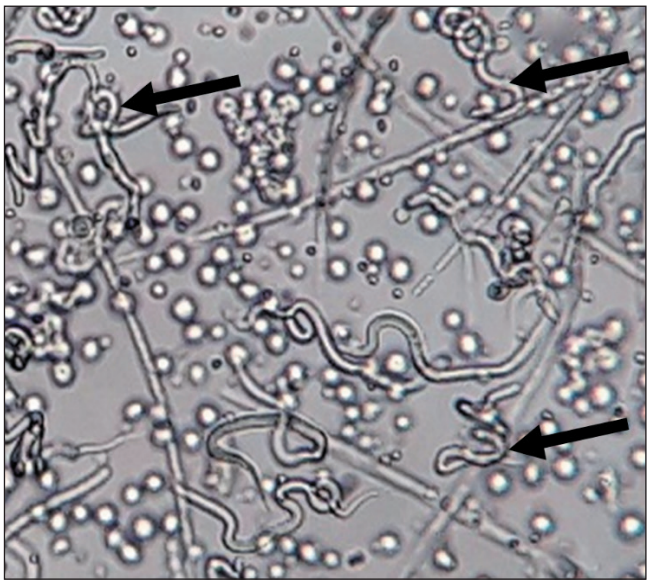

Figure 5. Mf as observed under microscope.

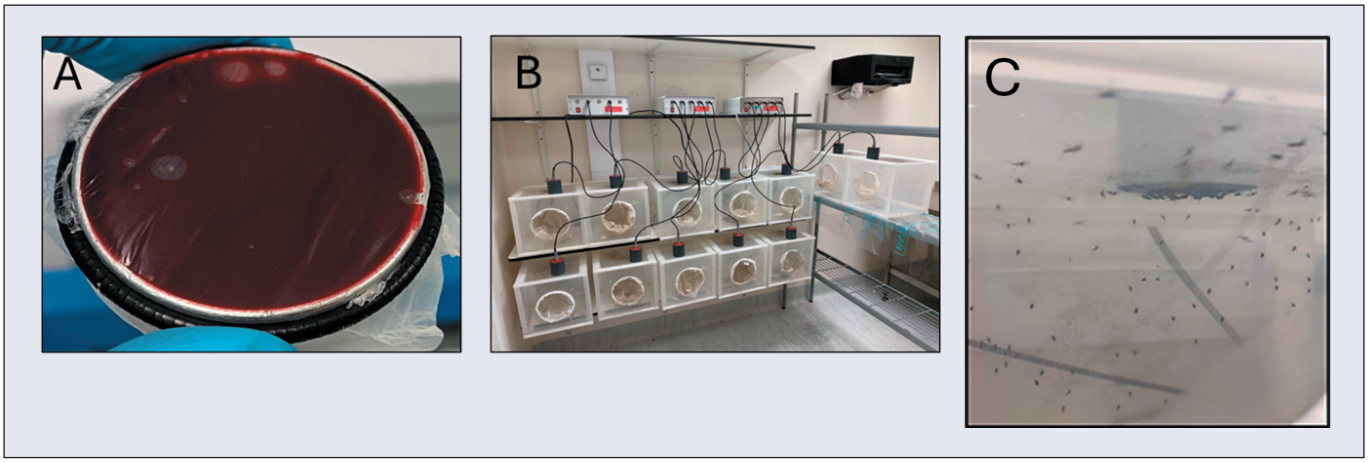

Figure 6. Infecting *Aedes aegypti* using the Hemotek® artificial feeding system.

mosquitoes are maintained for 14 days with daily 10% sugar-water feeding to allow *B. malayi* to develop into the infectious third-stage larvae (L3).

The infected blood meal is placed in feeding discs with a parafilm membrane and secured with a rubber seal (A). Discs are attached to the Hemotek® feeding system and placed on top of multiple cages of *Aedes aegypti* (B, C). Mosquitoes are fed for up to 2 hours.

L3 isolations from mosquitoes

After 14 days all mosquitoes fed on the infected blood meal are chilled for 10 minutes at 4°C (using a walk-in fridge) and combined into a single cage. Alternatively, a rapid 60 second incubation in a -20°C freezer can be utilised. Approximately 400 mosquitoes are then placed onto a glass plate L270 x W220mm. Using a 5ml Pasteur pipette, 15mls of warm 37°C modified RPMI containing 1% penicillin-streptomycin media is added to the chilled mosquitoes. A 25ml serological pipette is then used to crush the mosquitoes by gently rolling over them in a back-and-forth motion to release the L3. The crushed mosquitoes are washed off the plate using 100mls of warm modified RPMI media. The plate is held at an angle and the mosquitoes washed into a 500ml glass beaker. Four sets of Baermann's[15] apparatus are set up (Figure 7), which consists of a 150mm glass funnel attached to a clamp stand with a 75µm stainless steel filter placed in the funnel. A rubber tube is connected to the funnel and clamped off approximately four inches from the end using a dialysis clamp. Warm modified RPMI media (up to 400ml) is poured through the filter into the funnel and any bubbles that have formed are removed from the filter. The crushed mosquitoes are then poured into the filter and the L3 larvae allowed to descend for a maximum of two hours. Following this filtration, a second clamp is placed approximately 4 inches above the bottom clamp which is removed allowing the media containing the L3 larvae to be released into a 90mm petri dish.

Infected mosquitoes are placed into the sieve with the warmed modified RPMI media and the L3 are allowed to settle at the bottom of the tubing for up to 2 hours. A second clip is used to pinch off the tubing approximately 4 inches above the bottom green clip which can then be removed allowing retrieval of L3 into petri dishes.

Re-infection of Mongolian gerbils

The L3 are collected from the petri dishes using a 5ml sterile pasteur pipette and transferred into a 50ml sterile falcon tube and 30mls of RPMI is added to the tube with 4x penicillin-streptomycin and 4x amphotericin B. The falcon tube is placed into a 37°C incubator to keep the L3 motile for a period of 30 minutes to let the L3 sink to the bottom of the tube. After this the top 30mL of media is removed with a sterile pipette and replaced with fresh RPMI with 4x penicillin-streptomycin and 4X amphotericin B to wash the L3. The tube is transferred back to the incubator for another 30 minutes. This

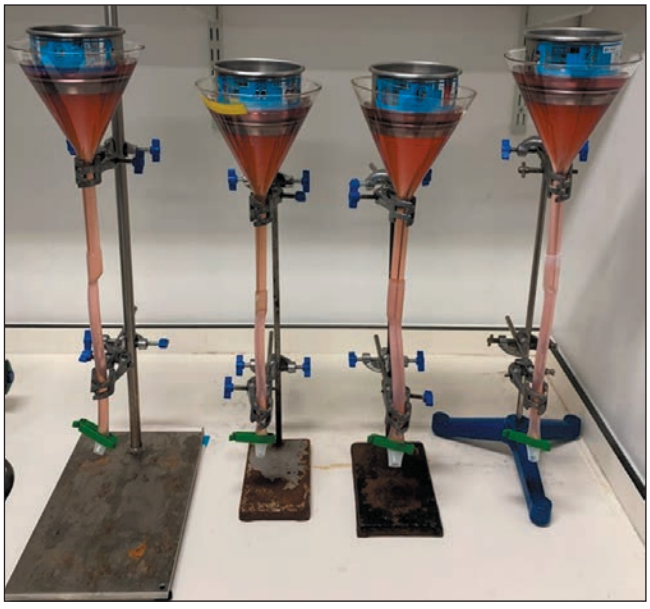

Figure 7. Set up of Baermann's apparatus.

process is repeated 3 times in total using sterile pre-warmed media. Once this is done the L3 are checked to make sure they are settled before removing top media. One final wash is then required with 2x penicillin-streptomycin and 2x amphotericin B and the contents are then poured onto a sterile 90mm petri dish.

L3 are then counted, via microscopy, by pipetting approximately 20 L3/20µl into individual wells of a 96 well plate to a total of 100 L3/100µl per well. Using two 1ml syringes attached to a 27G x ¾mm needle, 200 L3 are loaded into each syringe for a total number of 400 L3, which is the optimal number needed for reinfection into a gerbil. Gerbils are anaesthetised with isoflurane and the L3 injected into the peritoneal cavity of 4 to 6 months old male gerbils weighing 80 to 100g each, thus starting the life cycle for *Brugia malayi*. All experiments are approved by the ethical committees of the University of Liverpool and LSTM and conducted under Home Office Animals (Scientific Procedures) Act 1986 (UK) requirements. Animal experiments were undertaken in accordance with NC3Rs ARRIVE guidelines.

Results

Mongolian gerbils (*Meriones unguiculatus*), serve as a well-established surrogate laboratory host for maintaining the *B. malayi* life cycle *in vivo*. This model is vital for producing multiple life-cycle stages (Figure 8) that are used for a wide range of experimental investigations into parasite biology and therapeutic testing.

Drug discovery and screening can be categorised into direct-acting drugs including compounds that directly kill or inhibit development into adult worms, or indirect-acting drugs, which target *Wolbachia* bacterial endosymbionts, that are critical for the parasite's development and survival.[16] Anti-*Wolbachia* drugs can target L3s which arrest development of the parasite at the early fourth stage delivering prophylactic therapy, which is important for Heartworm (*Dirofilariasis*) drug applications.[17,18] These larval screens can be used also as a rapid screen to select potential macrofilaricidal therapies; those that target adult worms. These therapies are urgently needed to complement existing microfilaricidal treatments and programmes such as mass drug administration with anthelminthic drugs, ivermectin, albendazole and diethylcarbamazine.[16] With the help of animal-based research and imaging technologies[19] (Figure 9) L3 larvae can be used to initiate infections in preclinical filariasis mouse models, which can then be studied over time to evaluate disease progression and monitor therapeutic responses. Advanced tools such as ultrasound bioimaging are increasingly employed in these models to non-invasively monitor worm burden, providing real-time insights into worm location, movement and response to treatments[20] (Figure 10). This imaging technology allows

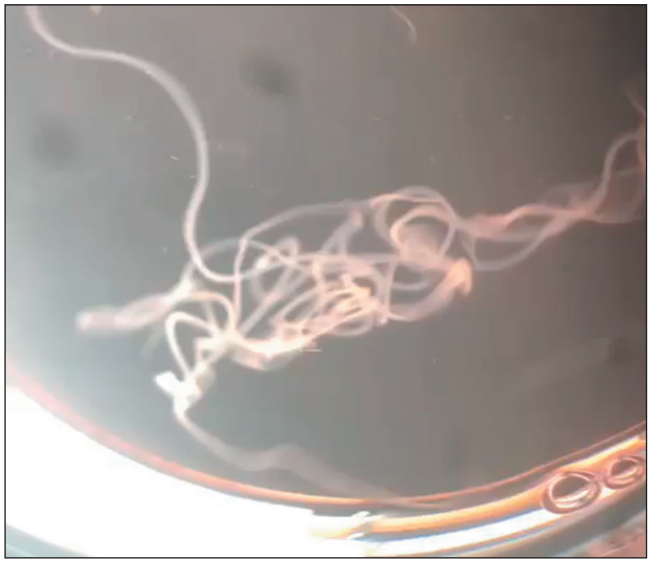

Figure 8. Adult worms from gerbil dissection for experimental use.

Figure 9. Alexa fluor 546 labelled *B. malayi* L3 larvae. An L3 larvae stained with Alexa fluor 54619 before injection into the hind leg lymphatic system of a C57BL/6J Prox-1GFP mouse. Scale bar = 20µm.

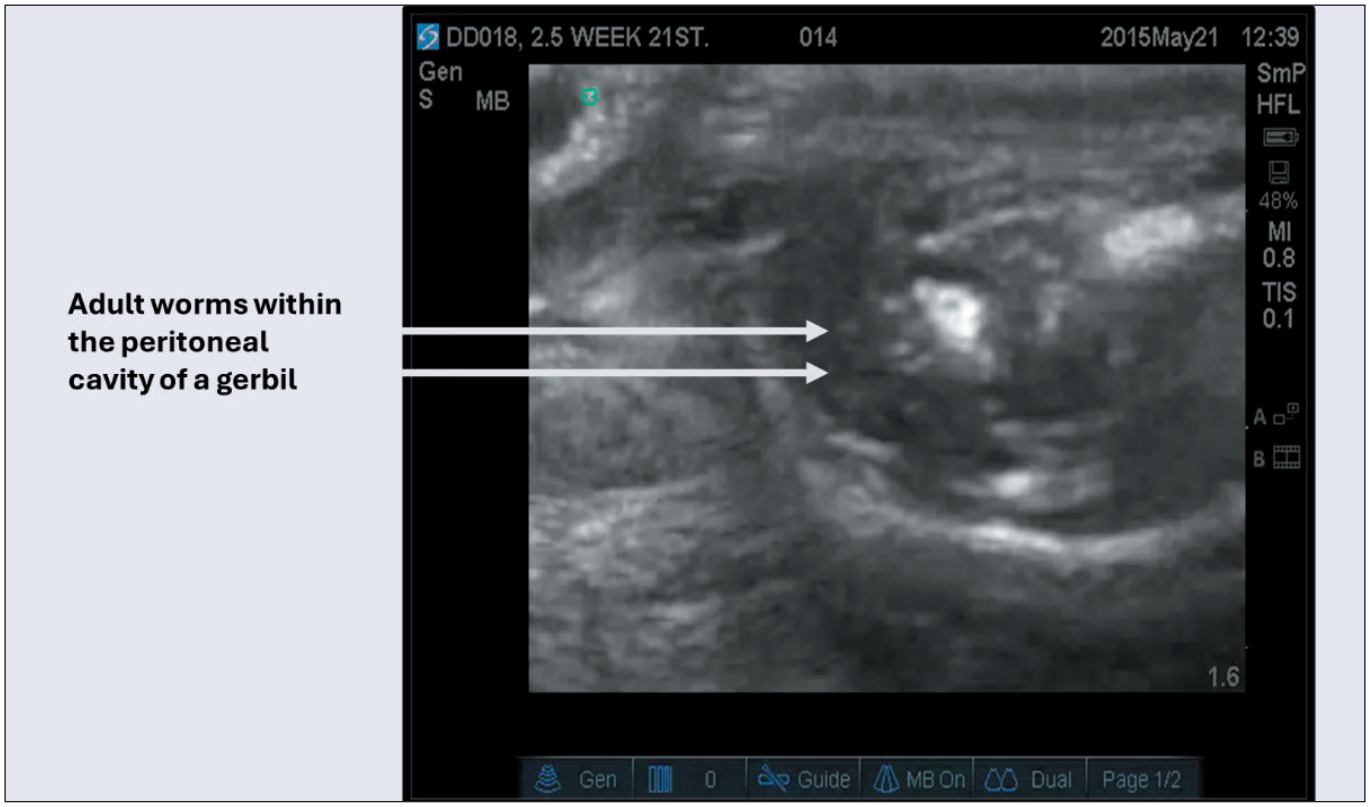

Figure 10. Still taken from an ultrasound video of an infected gerbil showing adult worms in the peritoneal cavity.

researchers to track treatment efficacy longitudinally, reducing the need for terminal procedures. This is a refinement in animal model usage following the 3Rs (Replacement, Reduction, Refinement) in animal research ethics. Gerbils are only imaged to confirm the presence of parasitic worms present in the abdominal cavity of a gerbil for treatments in preclinical drug screens.

This is not carried out on a routine basis, only when working on drug screen tests. All gerbils are imaged under continuous anaesthesia when the study is conducted. The abdominal region of the gerbil is shaved and then imaged by ultrasonography (USG) using a Sonosite® MTurbo® 8.5 Mz linear probe, for 10–15 minutes to confirm presence of parasitic worms.

Discussion

The use of adult male gerbils in *B. malayi* research provides a unique and valuable model for studying LF. However this model also presents specific biological, ethical and welfare considerations that must be carefully monitored.[21]

Gerbils (*M. unguiculatus*) are recognised as the lowest outbred vertebrate species that can be reliably infected with *B. malayi*. Research has shown that male gerbils are more susceptible to *B. malayi* infection than females.[4,22,23] Using males thus improves infection rates and parasite recovery, contributing to more consistent data and reducing the number of animals needed per experiment.

A critical feature of this model is that male gerbils can tolerate high parasite burdens in the peritoneal cavity with only rare incidents of clinical disease or overt pathology.[21] This is particularly useful for long-term studies, as it allows the infection to persist long enough to study the parasite's life cycle and host responses while minimising suffering.

To maintain Animal Welfare standards and comply with the 3Rs ethical research guidelines, infection studies in gerbils are limited to a duration of approximately 18 months, with a maximum lifespan of 24 months. These limits are imposed to prevent Animal Welfare issues associated with ageing, such as reduced organ function, chronic illness and increased sensitivity to stress.

By minimising confounding variables in experimental outcomes that could be influenced by age-related immunosenescence will further enhance Animal Welfare, in addition to ensuring humane endpoints are applied before animals' experience a decline in quality of life.

The low frequency of overt disease in male gerbils allows infections to proceed without the animals experiencing significant distress but still requires careful monitoring using regular health checks, weight tracking and behavioural observations.[21] Additionally, infection studies should be designed with predetermined humane endpoints and animals showing signs of discomfort or deterioration must be euthanised promptly.

The life cycle complexity of *Brugia malayi*, requiring both a mammalian and an insect host, presents significant challenges for *in vitro* cultivation of L3 to reproductively mature adults outside the host. While current co-culture approaches have had limited success,[24] emerging technologies such as organoid systems could provide improved *in vitro* culture systems.

The current approach of using gerbils to maintain the *Brugia malayi* life cycle reflects standard practice in filarial research, where animals are essential for supporting the maturation and reproduction of the parasites. However the exploration of non-invasive imaging modalities, such as ultrasound, offers a forward-thinking and welfare-conscious strategy to refine the use of animals and minimise harm, in line with the principles of the 3Rs.

The use of ultrasound imaging represents a major refinement in our protocol, offering several important advantages such as enabling longitudinal monitoring of worm development in the peritoneal cavity, allowing us to observe the growth, movement and location of adult worms over time. We are also able to assess and identify worm viability and burden without euthanising the host. This reduces stress and procedural risks associated with lavage. By imaging the same animal at multiple time points, unnecessary culling can be avoided, thus reducing the number of gerbils required to maintain the life cycle and for experimental studies.

The long-life cycle of *B. malayi* and the complex host-specific requirements for parasite development make the use of live vertebrate models, specifically gerbils, currently unavoidable in sustaining and studying the parasite. We and others in the filariasis community have used this model to make several significant advances in the biology of filarial nematodes. The discovery of the role of the essential bacterial symbiont *Wolbachia,* in parasite development, fecundity and survival[25,26,27,28] and exploiting the symbiotic relationship for curative therapy.[16,29,30,31,32] Our most recent discovery is that *B. malayi* hosts another type of microbiota, a single-stranded RNA virus, BMRV1, which elicits an antibody response from infected gerbils.[33]

Conclusion

Here we describe the maintenance of *B. malayi* in Mongolian gerbils and *A. aegypti* mosquito vectors, which serves as a propagation system and model for parasitic human filarial nematodes. Whilst no current alternatives for *in vitro* maintenance of the parasite's life cycle are available, we describe improvements in non-invasive imaging for monitoring parasite motility and viability *in vivo*.

Acknowledgments

The authors would like to thank the entire team within the UoL animal unit (BSU) for their expertise in Animal Welfare and the careful monitoring of the animals involved in this research. We would also like to thank Dr. Clair Rose for her help with the manuscript.

References

1 **Cano, J., *et al.*,** *The global distribution and transmission limits of lymphatic filariasis: past and present.* Parasite Vectors, 2014. 7: p. 466.

2 **Taylor, M.J., A. Hoerauf and M. Bockarie**. *Lymphatic filariasis and onchocerciasis.* Lancet, 2010. 376 (9747): p. 1175-85.

3 WHO. *Lymphatic filariasis (Elephantiasis).* 2025 2024; Available from: https://www.who.int/health-topics/lymphatic-filariasis#tab=tab_1

4 **Ash, L.R. and J.M. Riley.** *Development of subperiodic Brugia malayi in the jird, Meriones unguiculatus, with notes on infections in other rodents.* J Parasitol, 1970. 56(5): p. 969-73.

5 **Macdonald, W.W.** *The genetic basis of susceptibility to infection with semi-periodic Brugia malayi in Aedes aegypti mosquito* Ann. Trop. Med. Parasitol, 1962. 56: p. 373–382.

6 **Macdonald, W.W. and P.M. Sheppard.** *Cross-over Values in the Sex Chromosomes of the Mosquito Aedes Aegypti and Evidence of the Presence of Inversions.* Ann Trop Med Parasitol, 1965. 59: p. 74-87.

7 **Macdonald, W.W.** *The selection of a strain of Aedes aegypti susceptible to infection with semi-periodic Brugia malayi.* Ann. Trop. Med. Parasitol, 1962. 56: p. 368-372.

8 **Kuno, G.** *Early history of laboratory breeding of Aedes aegypti (Diptera: Culicidae) focusing on the origins and use of selected strains.* J Med Entomol, 2010. 47(6): p. 957-71.

9 Bugdorm. *Insect rearing cages*. 2025; Available from: https://shop.bugdorm.com/product_info.php?products_id=1&currency=GBP&ceid=18102c58ecaa77f82b9f05fd03dcf887

10 **Batchelder, M., *et al.*** *Gerbils.* The Laboratory Rabbit, Guinea Pig, Hamster, and Other Rodents, 2012: p. 1131-55.

11 **Faye Schrater, A., Spielman, A. and Piessens, W.F.** *Predisposition to Brugia Malayi Microfilaremia in Progeny of Infected Gerbils.* The American Journal of Tropical Medicine and Hygiene, 1983. 32(6): p. 1306-1308.

12 Labs, J. *GERBIL Meriones Unguiculatus*. 2025; Available from: https://janvier-labs.com/en/fiche_produit/gerbil/

13 Tecniplast. *Green Line GM1800 IVC*. 2025; Available from: https://www.tecniplast.it/en/product/double-decker.html

[14] **Griffiths, K.G., *et al.*** *Using an intravenous catheter to carry out abdominal lavage in the gerbil.* Lab Anim (NY), 2010. 39(5): p. 143-8.

[15] **Baermann, G.** *Eine einfache Methode zur Auffindung von Ankylostomum (Nematoden) Larven in Erdproben.* Geneeskundig Tijdschrift voor Nederlandsch-Indie, 1917. 57: p. 131-137.

[16] **Johnston, K.L., *et al.*** *Anti-Wolbachia drugs for filariasis.* Trends Parasitol, 2021. 37(12): p. 1068-1081.

[17] **Marriott, A.E., *et al.*** *Dirofilariasis mouse models for heartworm preclinical research.* Frontiers in Microbiology, 2023. Volume 14 - 2023.

[18] **Turner, J.D., *et al.*** *Novel anti-Wolbachia drugs, a new approach in the treatment and prevention of veterinary filariasis?* Vet Parasitol, 2020. 279: p. 109057.

[19] **Furlong-Silva, J., *et al.*** *Tetracyclines improve experimental lymphatic filariasis pathology by disrupting interleukin-4 receptor-mediated lymphangiogenesis.* J Clin Invest, 2021. 131(5).

[20] **Marriott, A.E., *et al.*** *Validation of ultrasound bioimaging to predict worm burden and treatment efficacy in preclinical filariasis drug screening models.* Scientific Reports, 2018. 8(1): p. 5910.

[21] **Chambers, C.A., *et al.*** *An Overview of Management Considerations for Mongolian Gerbils (Meriones unguiculatus), Cats (Felis catus), and Dogs (Canis familiaris) as Hosts for Brugia Infection.* Comp Med, 2024. 74(3): p. 142-147.

[22] **McCall, J.W., M.J., Hyong-Sun A., Thompson PE.** *Mongolian jirds (Meriones unguiculatus) infected with Brugia pahangi by the intraperitoneal route: a rich source of developing larvae, adult filariae, and microfilariae.* J. Parasitol, 1973. 59(436).

[23] **Ash, L.R.** *Chronic Brugia pahangi and Brugia malayi infections in Meriones unguiculatus.* 1973. 59(3): p. 442-447.

[24] **Marriott, A.E., *et al.*** *A mouse infection model and long-term lymphatic endothelium co-culture system to evaluate drugs against adult Brugia malayi.* PLoS Negl Trop Dis, 2022. 16(6): p. e0010474.

[25] **McGarry, H.F., Egerton, G.L. and Taylor, M.J.** *Population dynamics of Wolbachia bacterial endosymbionts in Brugia malayi.* Mol Biochem Parasitol, 2004. 135(1): p. 57-67.

[26] **Foster, J., *et al.*** *The Wolbachia genome of Brugia malayi: endosymbiont evolution within a human pathogenic nematode.* PLoS Biol, 2005. **3**(4): p. e121.

[27] **Landmann, F., *et al.*** *Anti-filarial activity of antibiotic therapy is due to extensive apoptosis after Wolbachia depletion from filarial nematodes.* PLoS Pathog, 2011. 7(11): p. e1002351.

[28] **Quek, S., *et al.*** *Wolbachia depletion blocks transmission of lymphatic filariasis by preventing chitinase-dependent parasite exsheathment.* Proc Natl Acad Sci USA, 2022. 119(15): p. e2120003119.

[29] **Townson, S., *et al.*** *Antibiotics and Wolbachia in filarial nematodes: antifilarial activity of rifampicin, oxytetracycline and chloramphenicol against Onchocerca gutturosa, Onchocerca lienalis and Brugia pahangi.* Ann Trop Med Parasitol, 2000. 94(8): p. 801-16.

[30] **Halliday, A., G.A., Tyrer, H.E., *et al.*** *A murine macrofilaricide pre-clinical screening model for onchocerciasis and lymphatic filariasis.* Parasit Vectors., 2014 Oct 24;. 7:(472).

[31] **Taylor, M.J., *et al.*** *Preclinical development of an oral anti-Wolbachia macrolide drug for the treatment of lymphatic filariasis and onchocerciasis.* Sci Transl Med, 2019. 11(483).

[32] **Hegde, S., *et al.*** *Combinations of the azaquinazoline anti-Wolbachia agent, AWZ1066S, with benzimidazole anthelmintics synergise to mediate sub-seven-day sterilising and curative efficacies in experimental models of filariasis.* Front Microbiol, 2024. 15: p. 1346068.

[33] **Quek, S., *et al.*** *Diverse RNA viruses of parasitic nematodes can elicit antibody responses in vertebrate hosts.* Nat Microbiol, 2024. 9(10): p. 2488-2505.