# THE SPRAGUE DAWLEY RAT AS A PRODUCTION MODEL FOR *BRUGIA PAHANGI*

by

KATELIN ELIZABETH GREENWAY

(Under the Direction of Andrew R. Moorhead)

## ABSTRACT

The Mongolian jird (*Meriones unguiculatus*) is the preferred rodent model for production of *Brugia pahangi* parasites. When inoculated intraperitoneally with *B. pahangi* L3, the parasites can reach adulthood and reproduce while remaining contained within the jird's abdominal cavity. Although the jird is a highly successful model, it presents challenges in the form of high cost, small size, and their status as a USDA regulated species. In this study we investigated the potential of an immune suppressed Sprague Dawley rat to serve as a production model for *B. pahangi*. We did this by assessing larval recovery rates in rats euthanized 14 days after inoculation with *B. pahangi* L3. We also performed differential cytologies of peritoneal fluid to determine what cell populations were present in infected animals vs naïve animals. Minimal larval recovery results indicate that the immune suppressed Sprague Dawley rat is not an ideal candidate for *B. pahangi* production.

INDEX WORDS: *Brugia pahangi*, Sprague Dawley rat, Mongolian jird, Filariasis

# THE SPRAGUE DAWLEY RAT AS A PRODUCTION MODEL FOR *BRUGIA PAHANGI*

by

KATELIN ELIZABETH GREENWAY

B.S., University of Georgia, 2018

A Thesis Submitted to the Graduate Faculty of the University of Georgia in Partial Fulfillment of the Requirements for the Degree

MASTER OF SCIENCE

ATHENS, GEORGIA

2024

THE SPRAGUE DAWLEY RAT AS A PRODUCTION MODEL FOR *BRUGIA PAHANGI*

by

KATELIN ELIZABETH GREENWAY

| | |
|---|---|
| Major Professor: | Andrew R. Moorhead |
| Committee: | Michael Dzimianksi |
| | Timothy Geary |

Electronic Version Approved:

Ronald Walcott
Dean of the Graduate School
The University of Georgia
December 2024

DEDICATION

To my family and friends, whose unwavering support and encouragement has been my guiding light. This work is a testament to your belief in me.

# ACKNOWLEDGMENTS

I would like to express my deepest gratitude to the following individuals who have supported me throughout my academic and professional career:

First and foremost, I am profoundly thankful to my major advisor, Dr. Andy Moorhead, whose guidance, expertise, and encouragement have been invaluable. His insightful feedback and unwavering belief in my abilities pushed me to strive for excellence. My committee members, Dr. Mike Dzimianski and Dr. Tim Geary, for their constructive criticism, advice, and support during this process. My lab colleagues, Elyssa Campbell, Leonor Sicalo Gianechini, Angela Tolbert, Tanya Cooper, Yi Chu, Chris Evans, Liz Boudreau, Sam Hogan, and Izzy Morgan for their never-ending willingness to offer a helping hand or a listening ear when it was needed. My family, for their encouragement and consistent motivation to be the best that I can be. To Flo, Toni, & Georgia, who remained a steady source of emotional support at the end of a long day. The Filariasis Research Reagent Resource Center and associated Scientific Advisory Committee for providing the inspiration for this research and the resources to complete it.

I am also thankful for everyone who has contributed to my journey, whether directly or indirectly. Your support has been instrumental in my achievements, and I look forward to carrying these experiences into the future.

TABLE OF CONTENTS

ACKNOWLEDGEMENTS ..... v

LIST OF TABLES ..... vii

LIST OF FIGURES ..... viii

CHAPTER

1 Introduction and literature review

Overview of Lymphatic Filariasis ..... 1

Elimination, Diagnosis, and Treatment ..... 3

Causative agents of Lymphatic Filariasis ..... 5

Common *Brugia* spp. Animal models for lymphatic filariasis research ..... 6

The Filariasis Research Reagent Resource Center (FR3) ..... 8

Promising *Brugia pahangi* rodent models ..... 9

Anthelmintic laboratory animal model for heartworm ..... 16

Immune response of rats ..... 17

References ..... 19

2 Materials and Methods ..... 23

3 Results ..... 28

4 Discussion ..... 35

5 Conclusions ..... 40

LIST OF TABLES

Table 1.1: FR3 parasite contributions .....13

Table 1.2: Susceptibility of conventional and athymic mouse strains to infection with *B. pahangi* .....16

Table 3.1: Larval recovery pilot data of *B. pahangi* L3, L4, and adult worms.....34

Table 3.2: Larval recovery .....36

LIST OF FIGURES

Figure 1.1: *B. malayi* life cycle ..... 7

Figure 2.1: Experimental design ..... 31

Figure 3.1: Larval recovery of *B. pahangi* L3, L4, and adult worms ..... 33

Figure 3.2: Total recovery of larvae ..... 35

Figure 3.3: Peritoneal cell populations of experimentally infected rodents ..... 37

Figure 3.4: Individual cell populations of experimentally infected rodents ..... 3

# CHAPTER 1

# INTRODUCTION AND LITERATURE REVIEW

## Overview of lymphatic filariasis

Lymphatic filariasis (LF) is one of 20 diseases collectively referred to as neglected tropical diseases (NTDs). LF threatens over 657 million people across 39 tropical and sub-tropical countries. *Wuchereria bancrofti* is the causative agent of 90% of LF cases while *Brugia malayi* comprises the other 10% [1]. *Wuchereria bancrofti* and *B. malayi* are filarial nematodes transmitted through a mosquito vector. When a mosquito takes a blood meal from an infected host, it ingests microfilariae (mf). Over the course of approximately two weeks, the mf mature inside the mosquito, undergoing two molts, eventually migrating to the proboscis as infective third-stage larvae (L3). When the infected mosquito takes another blood meal, the L3 are deposited on the skin and enter the puncture site after the mosquito removes its stylet. They migrate to the lymphatic system where they finish maturing to adulthood and begin producing mf to be dispersed throughout the bloodstream (Figure 1.1). The time from infection to patency for a lymphatic filariasis infection can be as early as 3 months [2]. Adult worms can live for 6-8 years and produce millions of mf during that time [1].

The individuals most at risk of infection live in tropical or sub-tropical endemic regions. Contracting the disease usually requires multiple bites from infected mosquitoes over several months or years, so brief travelers to endemic regions, meaning individuals that are just passing through or are only in the area for a short period of time (days to weeks), are generally not at risk

[3]. Lymphatic filarial infections can be asymptomatic, acute, or chronic. Acute episodes occur as a result of an infected individual's immune response to the antigens released when adult worms die and are typically associated with fever, painful lymphadenopathy, and a dry cough or wheeze. Chronic infections are associated with severe lymphedema or hydrocele caused by long-term damage to the lymphatics. Most infections are asymptomatic, despite still having a negative impact on the afflicted person's lymphatic system, kidneys, and immune function, while also contributing to the continued spread of the disease [1]. Symptomatic infections cause lymphedema, hydrocele, hardening or thickening of the skin, respiratory symptoms, and secondary bacterial skin infections [3]. Apart from the physical symptoms associated with the disease, many people suffer from negative social and mental consequences as well. Because many endemic locations are rural, agricultural communities that rely on intense physical labor, severe lymphedema and bacterial infections lead to a loss of income-earning opportunities and increased medical expenses for the afflicted and their caregivers. These effects can also contribute to low mental health rates and carry a negative social stigma. Though this disease is not associated with high mortality rates, it is known for high morbidity rates [1].

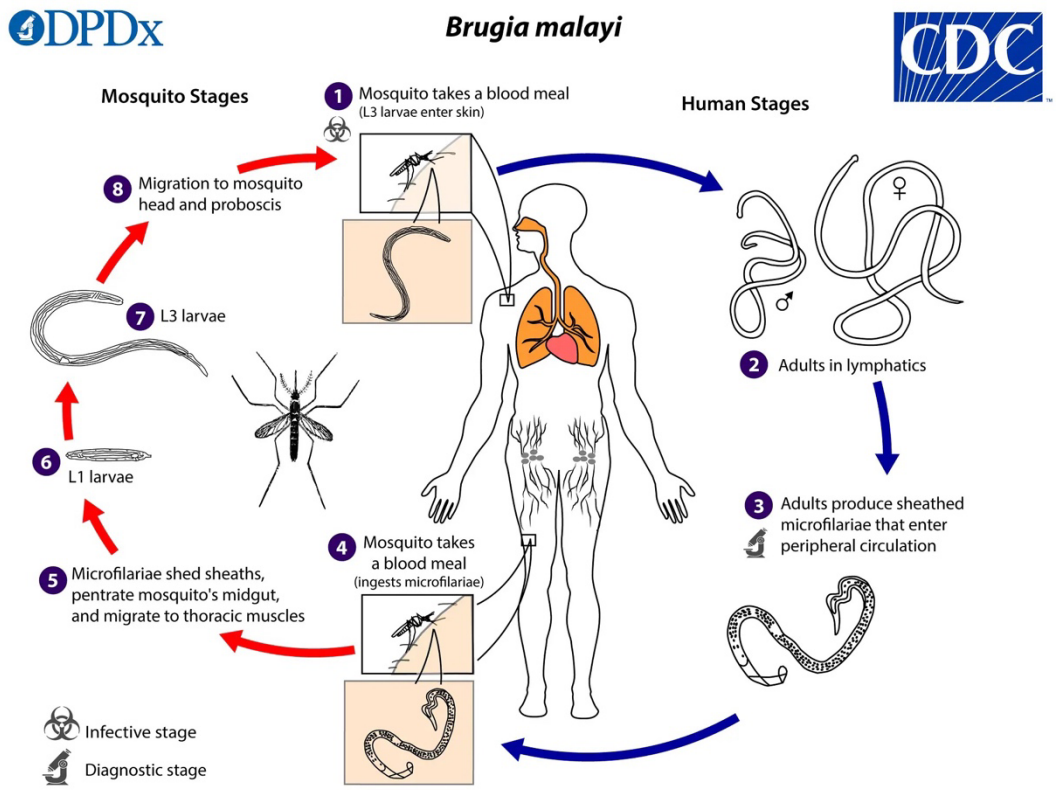


**Figure 1.1**
*Brugia malayi* life cycle
*Note:* Though *B. pahangi* was used for this study, the life cycle is almost identical to that of *B. malayi* depicted above.

## Elimination, Diagnosis, and Treatment

The Global Program for the Elimination of Lymphatic Filariasis (GPELF) was established in 2000 by the World Health Organization (WHO) with the purpose of stopping the spread of LF and alleviating the suffering of those infected. The main method used to stop the spread of this disease is mass drug administration (MDA). As of 2023, LF has been officially eliminated in 20 previously endemic countries. Fourteen countries are currently under surveillance to demonstrate that elimination has been achieved. Currently, 39 countries continue to use MDA and preventive chemotherapy, while six of those countries have not yet implemented MDA. In 2020, GPELF set a goal for 80% of endemic countries to have met criteria for validation of elimination of the

disease as a public health concern, 100% of endemic countries to be implementing post-MDA or post-validation surveillance, and for no endemic countries to still require MDA by 2030. For a country to have met criteria for validation of elimination of LF as a public health concern, they need to have sustained infection rates below target thresholds for at least 4 years post-MDA and provide essential care packages to all areas with known patients. Based on the socio-economic impacts described previously, elimination of LF could lead to an overall reduction in poverty levels in impacted areas [1].

Due to the presence of mf in the bloodstream of infected individuals, examining a blood sample microscopically is the standard diagnostic method for LF. In some cases, an antibody test may also be used for diagnosis of a current infection. Treatment of LF typically comes in the form of diethylcarbamazine (DEC) administration. DEC can be given as a single administration or daily for a total of 12 days. This treatment is only effective in killing immature and adult worms, but is not useful for alleviating chronic symptoms. Treatment for chronic symptoms such as elephantiasis or hydrocele focus more on learning to manage symptoms and prevent secondary bacterial infections [4].

The WHO eradication efforts primarily focus on education and stopping the spread of LF through MDA and surveillance in endemic regions. Recommended MDA regimens are determined based on the absence or presence of other filarial diseases in the region receiving MDA. In countries co-endemic with loiasis, it is recommended to administer albendazole twice annually. Based on information obtained from the Expanded Special Project for Elimination of Neglected Tropical Diseases (ESPEN), ivermectin is not a recommended treatment in countries with loiasis due to the potential for severe adverse reactions that can occur in individuals with high *Loa loa* infections. Twice annual albendazole can reduce the microfilarial burden in those

with LF, thus reducing the risk of LF transmission without the adverse reactions [5]. According to the CDC, in countries with onchocerciasis, albendazole is recommended in addition to ivermectin annually. DEC is not a recommended treatment in countries co-endemic with onchocerciasis due to the potential for blindness or severe skin disease [6]. Finally, in countries without onchocerciasis, DEC, albendazole, and ivermectin are recommended for annual MDA [1]. The CDC recommends avoiding areas with large populations of mosquitoes, especially in the evenings when the mosquito species responsible for transmitting filarial parasites are more likely to bite. Other methods of prevention include DEET-based insect repellant, wearing loose-fitting and long-sleeved clothes, and to keep doors and windows closed or covered with screens [3].

**Causative agents of lymphatic filariasis**

As mentioned previously, *W. bancrofti* is the causative agent of approximately 90% of lymphatic filariasis cases while *B. malayi*, found in China, India, Malaysia, the Philippines, Indonesia, and some Pacific Islands, makes up the other 10%. A few cases are attributed to *Brugia timori,* which is only found on the Timor Island of Indonesia [1, 7]. Though *W. bancrofti* is the primary species responsible for LF cases, *B. malayi* and *B. pahangi* are the primary research models for studying this disease. This is because humans are the definitive host for *W. bancrofti*, making it difficult to conduct research on the parasite in a controlled setting. A non-human primate model, the Indian leaf monkey (*Presbytis entellus*), can be used to produce and study *W. bancrofti* [8]. However, maintaining and working with non-human primates in a controlled setting comes with a host of health and safety challenges that the typical research laboratory is not equipped to handle. *Brugia pahangi* is another mosquito-borne filarial

nematode closely related to *B. malayi*. It can be found in Southeast Asia, specifically Malaysia and Thailand [9]. Based on the genomic analysis and gene annotation, *B. pahangi* and *B. malayi* have 89.6% orthologous genes [10]. This similarity allows scientists to use both parasites to study human filariasis. *Brugia malayi* and *B. pahangi* can be easily maintained in a variety of small animal models including dogs, cats, and rodents. This allows scientists relatively easy access to the parasites and the ability to study them in a controlled environment with known infection dates, inoculum numbers, and disease progression.

## Common *Brugia* spp. animal models for lymphatic filariasis research

Animal models have contributed to a significant number of scientific advancements in the field of parasitology. These animal models can be highly beneficial in examining the details of host-parasite interactions, screening novel compounds or evaluating drug efficacy, vaccine development, basic biological understanding of parasite species, and many other areas of importance. The use of animals in research is highly regulated by the United States Department of Agriculture and the Public Health Service. Each research institution also has an oversight committee, the Institutional Animal Care and Use Committee or IACUC, comprised of at least five members that must include a veterinarian with laboratory animal experience, a practicing scientist with animal research experience, a nonscientist focusing on ethical or legal obligations, and a non-affiliated member. It is the responsibility of these individuals to review and grant or withhold approval of studies involving the use of animals [11].

### *The domestic dog (Canis lupus familiaris)*

The domestic dog is an ideal model for large-scale production of *Brugia* spp. for many reasons, namely the ease with which they can be handled, the high numbers of circulating mf,

and the relatively large volume of blood that can be obtained at one time from one animal. In a study published in 2022, 41% of dogs experimentally infected with *B. malayi* maintained microfilaremia for more than two years, making the dog model reasonably comparable to the domestic cat [12].

***The domestic cat (Felis catus)***

The domestic cat is one of the primary laboratory animal models for *B. malayi*. This is due to their relatively high infection rates and their ability to maintain high levels of mf after experimental infection [12, 13]. Cats have been the preferred small animal model because of the larger volume of microfilaremic blood that can be collected from them. Restraint for blood collection poses a risk of injury to research personnel due to the potential for bites and scratches, which can cause serious bacterial infections. According to the CDC, about 20-80% of cat bites and scratches become infected [14]. If infections are severe or go untreated for too long, hospitalization for intravenous antibiotic administration may be necessary. To minimize this risk of injury, cats often require sedation through the use of anesthetics for blood collection. The use of anesthetics is highly regulated and presents its own challenges related to acquisition and storage [12].

***The Mongolian gerbil (Meriones unguiculatus)***

The Mongolian gerbil (*Meriones unguiculatus*), commonly known as the jird, is the preferred laboratory host for *Brugia* spp. The jird is susceptible to subcutaneous infections of both *B. malayi* and *B. pahangi* with successful infections producing mf [15, 16, 17, 19]. Jirds are also susceptible to intraperitoneal infections of both previously mentioned *Brugia* species, which are useful for producing large quantities of adult worms and mf [17]. The jird currently serves as

one of the animal models for the production of *B. malayi* and *B. pahangi* at the NIH NIAID-funded Filariasis Research Reagent Resource Center (FR3) at the University of Georgia [18].

**The Filariasis Research Reagent Center**

The FR3 was established in 1969 at the University of Georgia to provide filarial parasite materials to labs around the world and develop and refine experimental filariasis protocols, most of which are still in use today. The FR3 is funded by the NIH NIAID Animal Models of Infectious Disease program. A Scientific Advisory Committee reviews FR3 performance annually and reports findings to the director and the NIAID to better understand the research community's needs and ensure they are being met. This committee is comprised of highly qualified members of the filariasis research community [18].

Currently, the FR3 uses the domestic dog, jird, and black-eyed Liverpool strain *Aedes aegypti* mosquito to maintain *B. pahangi* and *B. malayi*. The domestic cat was also used in the production of the *B. malayi* life cycle until the previously mentioned 2022 publication confirming the domestic dog as a suitable laboratory model for this species [12]. Maintaining these materials is costly, resource-intensive, and demands a large, specially-trained staff, which underscores the necessity for a central repository. Currently, the FR3 currently maintains two filarial species, *B. malayi* and *B. pahangi*. Since its inception, the FR3 has contributed a wealth of materials and knowledge to the filariasis research community and continues to be an irreplaceable resource [18] (Table 1.1). Since 2017, the FR3 has been acknowledged in at least 152 publications for providing resources and expertise.

**Table 1.1**
FR3 Parasite Contributions

| Parasite Species | Vector Species | Years Provided |
|---|---|---|
| *Litomosoides sigmodontis* | *Ornithonyssus bacoti* | 1969 - ~2000 |
| *Acanthocheilonema viteae* | *Ornithodoros tartakavskyi* | 1969 - ~2000 |
| *Dirofilaria immitis* | *Aedes aegypti* | 1970 - 2022 |
| *Brugia malayi* | *Aedes aegypti* | 1970 - present |
| *Brugia pahangi* | *Aedes aegypti* | 1970 - present |

## Promising *Brugia pahangi* rodent models

### *The golden hamster* (*Mesocricetus auratus*)

The golden hamster has been investigated as a potential model for filariasis studies. In a study published in 1975 by Malone & Thompson, golden hamsters were shown to develop patent infections after subcutaneous inoculation with *B. pahangi* L3 [20]. Adult worms were recovered at an average of 14% (after inoculation with 95 to 150 L3) from 11 hamsters between 105- and 195-days post infection. Scientists also recovered adult worms at a slightly higher average of 16% from eight hamsters between 23- and 45-days post infection. This study noted that 10 out of 12 hamsters that were allowed to carry their infections past 100 days developed low levels of microfilaremia. The worms harvested from these subcutaneously-infected hamsters were found in afferent or efferent regional lymph node vessels, heart, lungs, testes, epididymis, and spermatic cord with over half being collected from the latter three locations. Based on these findings, Malone & Thompson concluded that the golden hamster is only half as susceptible to subcutaneous infection compared to the jird. Malone & Thompson also investigated the intraperitoneal route of inoculation for the golden hamster. They found that worms were recoverable for only a short time, up to 3 weeks post-inoculation. These results led them to

conclude that the peritoneal cavity of the hamster is not a suitable environment for production of *B. pahangi* [20].

While these studies indicate that the hamster is not a suitable production model for *B. pahangi*, pathological findings suggest that it may be a suitable model for host-parasite relationship studies as well as a model for gross pathology of genital filariasis. Lesions in hamsters are consistent with those described in early filarial infections of humans and other experimentally infected animals, specifically genital lymphatic changes [20]. The hamster is also a suitable model for gross pathology because it is easier to visualize the pathological changes in peripheral and retroperitoneal lymphatic vessels and nodes of hamsters than in the jird due to their larger size [20].

***Mouse models***

Suswillo et al. (1980) conducted a study investigating the susceptibility of AKR, BALB/c, CBA/Ca, and T.O. strains of conventional mice and nude (athymic) mice to infection with *B. pahangi* [21] (Table 1.2). They found that all strains of mice were resistant to infection with *B. pahangi* L3 harvested from mosquitoes. However, when the conventional mice were infected intraperitoneally with L3, L4, or juvenile adult stages of *B. pahangi* worms transplanted from the jird peritoneal cavity, the worms continued to develop. They found that the BALB/c strain was the most susceptible and were able to recover male and female adult worms and mf after each infection with transplanted worms. Athymic mice were found to be more susceptible to infections with transplanted worms and the larvae developed to full maturity in most of the inoculated mice with 10 of 14 developing mf in the blood or peritoneal cavity. Ten mice had mf in their peritoneal cavity and eight had mf circulating in their blood. They recovered an average of 11.1% of transplanted worms from the peritoneal cavity of these athymic mice. When

inoculated subcutaneously, 2 of 6 athymic mice developed mf and adult worms were recovered at an average of 6.1%. The authors also compared susceptibility of thymic littermates of the athymic mice and found that they demonstrated higher levels of resistance than the other strains of mice used in the study. Only 18% of worms were recovered from thymic littermates implanted with adult *B. pahangi* worms from the jird peritoneal cavity, compared to 67% in athymic littermates. No worms or mf were recovered from jirds inoculated with L3 isolated from mosquitoes or L4 from the peritoneal cavity of the jird; their athymic littermates that had recovery rates of 4% and 23.9%, respectively [21].

**Table 1.2**
Susceptibility of conventional and athymic mouse strains to infection with *B. pahangi*

| Mouse Strain | Inbred vs Outbred | Genetic significance | Common Uses | Susceptibility to B. pahangi L3 harvested from mosquitoes | Susceptibility to *B. pahangi* L3 transplanted from jird peritoneal cavity | Susceptibility to *B. pahangi* L4 transplanted from jird peritoneal cavity | Susceptibility to *B. pahangi* juvenile adults transplanted from jird peritoneal cavity |
|---|---|---|---|---|---|---|---|
| AKR | Inbred | High leukemia incidence | Leukemia research | ✗ | ✗ | ✗ | ✓ |
| BALB/c | Inbred | Easily triggered Th2 cells | Immunology & Infectious Disease research | ✗ | Adult male and female worms recovered but no microfilariae | ✓ | ✓ |
| CBA/Ca | Inbred | Genetically homogeneous | Drug efficacy & toxicity, experiments needing consistent and predictable results | ✗ | Only adult males recovered, no microfilariae | Only adult males recovered, no microfilariae | ✓ |
| T.O. | Outbred | Exhibit the twister mutation, known for causing the mouse to tuck its head when picked up by the tail | Encephalomyelitis research | ✗ | ✗ | Only adult males recovered, no microfilariae | ✓ |
| Athymic | Inbred for initial experiments, outbred for later experiments | Lack a thymus; deficient in T cells | Cancer research, drug testing, & disease modeling | - | ✗ | ✓ | ✓ |

*Note:* Information in this table was compiled from [21]
An X indicates no adult worms or microfilariae were recovered at necropsy.
A check indicates both adult worms and microfilariae were recovered at necropsy.
A dash indicates the experiment was not conducted.

A study conducted by Vincent et al. (1982) further explored susceptibility in athymic mice and relative resistance in their phenotypically normal littermates [22]. Using the C3H/HeN strain, they found that athymic mice were highly susceptible to infection while their normal, or "hairy" littermates, were highly resistant. Infective larvae were harvested from infected mosquitoes and were injected either subcutaneously or intraperitoneally. In the nude mice, these larvae underwent the 3$^{rd}$ and 4$^{th}$ molts and matured to adults producing mf, though the adult female worms were smaller than usual; adult females were, on average, 2.74 cm in length while the average adult female of the same age recovered from a jird is approximately 3.3 cm in length. In athymic mice inoculated subcutaneously, adult worms were recovered at an average rate of 15%, with 75% of those found in the heart and lungs. Infections became patent as early as 50 days post infection. In athymic mice inoculated intraperitoneally, adult worm recoveries averaged between 16% and 51%. Those that were allowed to reach patency gave between 1.4 x $10^6$ and 1.9 x $10^6$ mf at necropsy. In contrast to these results, few worms were recovered after 40 days from the phenotypically normal littermates of the athymic mice and microfilaremia was not detected. These findings indicate that this model could be useful in protective immunity response studies between athymic and thymic mice with filarial infections [22].

***Rat models***

Rats have been a promising model in filariasis research for many years. In 1976, Fox and Schacher published a study comparing susceptibility to *B. pahangi* infection among several different syngeneic rat strains [23]. Lewis, Fisher 344, and Brown Norway rats were all highly susceptible to infection with *B. pahangi*, while Wistar Furth and ACI rats were moderately susceptible. Buffalo rats demonstrated high levels of resistance in their initial study, but were classified as moderately susceptible in further studies. All the forementioned rats were inoculated

subcutaneously with 50 L3. They were considered susceptible if adult *B. pahangi* worms were found at necropsy and if the rat developed microfilaremia. The authors determined that these models could provide a better means to study the cellular response to filarial infections [23].

Gusmao et al. furthered this research by evaluating the susceptibility of Lewis rats to subcutaneous *B. pahangi* infections along with a more in depth look at the immune response in these animals [24]. They observed patent infections in only two-thirds of the rats. In these animals, microfilaremia ranged from 2 to 796 mf/mL. The authors also stated that recovery of adult worms was extremely limited and recovery data were not reported. Blood leukocyte levels, antifilarial IgG and IgE antibodies, and lymphocyte responses were monitored for the first 50 weeks of infections. Each of these measurements remained nearly identical in rats that became infected and rats that resisted infection with the exception of specific IgE antibodies. Rats that were resistant to infection developed the specific IgE antibodies between five and eight weeks after inoculation, while these specific antibodies never developed in animals that became microfilaremic. The authors believed these findings were an indication that the specific IgE antibodies contributed to protecting the rats from filarial infection [24].

Cruickshank et al. evaluated infection rates among inbred PVG/c and athymic rats. After subcutaneous infection with *B. pahangi* L3, only 20 of 34 PVG/c rats developed adult worms or became microfilaremic, while all 30 athymic rats developed microfilaremia [25]. Authors noted that fertile adult worms were recovered from the lumbar lymphatics and hearts of both PVG/c and athymic infected rats upon necropsy. Intraperitoneal infection did not produce any mf or adult worms in any of the strains. The infection rate of athymic rats compared to the PVG/c rats led the authors to conclude that the immune response, and thus development of filarial infection, is T lymphocyte dependent. They concluded that athymic rats could be a model for filarial

immunopathology in human filariasis. They also concluded that PVG/c rats were a reproduceable and valuable model for studying tissue response, eosinophilia induction, and the role of cellular and humoral immunity in controlling filarial infection [25].

Vincent et al. evaluated the levels of *B. pahangi* mf production in subcutaneously inoculated male and female Lewis rats [26]. A higher proportion of male Lewis rats, 95%, demonstrated patency compared to 64% of female Lewis rats who developed patent infections, which were monitored for up to 420 days post-inoculation. Based on microfilaremia alone (recorded with a mean of 218 mf/0.25 mL blood), male Lewis rats are not as susceptible to infection as the Mongolian jird, but they do consistently become infected in a controlled setting and remain microfilaremic for more than a year. The authors concluded that based on greater male susceptibility, the male Lewis rat has the potential for use as a model for this aspect of human LF by examining the interaction between the immune system and the endocrine system [26].

Lawrence et al. evaluated levels of *B. pahangi* mf production based on various larval inoculation sizes [27]. When infected subcutaneously with 100 L3, 56% of PVG rats developed patent infections. This is consistent with previous studies referenced above in which approximately two-thirds of inoculated rats develop patency. Upon necropsy, only 1-3% of inoculated larvae had matured to adulthood. The authors hypothesized that the low levels of microfilaremia were due to the low probability of male and female worms settling in the same locations. When they increased the number of inoculated L3 from 100 to 500, 94% of the rats developed patent infections. The authors noted that all rats became amicrofilaremic and adult worms could not be recovered 420 days after inoculation [27].

Bell et al. evaluated susceptibility to *B. pahangi* infection among eight inbred strains of

rats: AO, PVG, WKA, LOU, BN, ACI, Lewis, and M520 [28]. All rats were inoculated subcutaneously with 200 L3. WKA rats had the highest percentage of mf production (73%) while BN rats had the lowest (23%). Suckling rats were highly susceptible while weanlings were intermediately resistant. As demonstrated in previous studies, females were highly resistant to infection and a high proportion of patent infections involved the testes and testicular lymphatics. Even in the most susceptible rats, 95% of administered larvae or developing L4 were cleared from the animal by 28 days post-inoculation and male worms demonstrated a shorter life span. The authors concluded that these rats demonstrated patency and infectivity patterns comparable to those seen in human infections and could be used as a model for human LF [28].

The multimammate rat (*Mastomys natalensis*) has been explored as a potential rodent model for lymphatic filariasis research [29]. The highest successful infection rate, meaning inoculated larvae matured to adulthood and began producing mf, was found to be in rats inoculated subcutaneously in the neck. They did note that in animals inoculated in the neck, most adult worms were collected from the heart and lungs while most worms could be collected from the testes and lymphatics of animals inoculated in the groin. These researchers concluded that the multimammate rat could be used as an alternative to the jird in LF studies, specifically in regards to subcutaneous infections [29].

**Anthelmintic laboratory animal model for heartworm**

There has been a recent increase in efforts to develop novel rodent models for producing and studying filarial nematodes as well as for screening novel compounds against these parasites. Zoetis, Inc. has patented such a model for the filarial nematode *Dirofilaria immitis*, canine heartworm, using the Sprague Dawley rat (*Rattus norvegicus*) [30]. The Sprague Dawley rat is a

multipurpose rodent model widely used for a variety of research needs including, but not limited to, toxicology, aging, and oncology.

According to the Zoetis patent, rats were fed a dietary mixture of hydrocortisone-21-acetate at 200 ppm for approximately 21 days (8 days pre-inoculation and 13 days post-inoculation). They were then fed the same dietary mixture at 50 ppm until necropsy and recovery of adult *D. immitis* worms at approximately 120 days post-infection [30]. This model was successful for conducting *in vivo* safety and efficacy studies for adult *D. immitis* in dogs as well as producing adult *D. immitis* worms for *in vitro* compound screening [30].

**Immune response of rats**

When faced with a new pathogen, a host can employ two immune responses. The innate immune system is the first line of protection. Examples of immune cells involved in this response are inflammatory cells, like macrophages and neutrophils, mast cells, and eosinophils [30]. If the innate immune system fails to eliminate the pathogen, the adaptive immune system begins to step in after about four to seven days [30]. The primary immune cells and proteins involved in the adaptive response are lymphocytes (either B lymphocytes or T lymphocytes) and antibodies [31].

In the following chapters, we investigated the immune suppressed Sprague Dawley rat model patented by Zoetis to create a novel model for large-scale production of *B. pahangi*. The development of a rat model for *B. pahangi* production would provide a larger, more cost efficient, non-USDA regulated alternative to the jird model. There is also a wealth of knowledge and resources available for studying cell populations and immune responses in rats that are not yet available for jirds. Historical literature suggests that some rat strains show promise as LF models, specifically those that are immune-suppressed or athymic. However, many of these

studies focused on subcutaneous infections whereas our primary interest is the intraperitoneal route of inoculation. In Chapter two, we explain the materials and methods used to conduct our studies. The results of the study are explained in Chapter three. In Chapter four, we discuss the implications of these results along with the limitations and challenges faced during the study. We also discuss the future directions this research could take and changes to experimental design that could be considered in future studies before concluding this thesis in Chapter five.

## References


1. (WHO), W.H.O. Lymphatic Filariasis. 2022; Available from: https://www.who.int/news-room/fact-sheets/detail/lymphatic-filariasis
2. Cross JH. Filarial Nematodes. In: Baron S, editor. Medical Microbiology. 4th edition. Galveston (TX): University of Texas Medical Branch at Galveston; 1996. Chapter 92. Available from: https://www.ncbi.nlm.nih.gov/books/NBK7844/
3. (CDC), C.D.C. About Lymphatic Filariasis. 2024; Available from: https://www.cdc.gov/filarial-worms/about/lymphatic-filariasis.html
4. (CDC), C.D.C. Treatment of Lymphatic Filariasis. 2024; Available from: https://www.cdc.gov/filarial-worms/treatment/index.html
5. (ESPEN), Expanded Special Project for Elimination of Neglected Tropical Diseases. Loiasis. Available from: https://espen.afro.who.int/diseases/loiasis
6. (CDC), C.D.C. Clinical Treatment of Loiasis. 2024; Available from: https://www.cdc.gov/filarial-worms/hcp/clinical-care/loiasis.html
7. Klion, Amy, *Lymphatic filariasis: Epidemiology, clinical manifestations, and diagnosis.* 2024; Available from https://www.uptodate.com/contents/lymphatic-filariasis-epidemiology-clinical-manifestations-and-diagnosis/
8. Misra, S., et al., *Experimental Transmission of Nocturnally Periodic Wuchereria bancrofti to Indian Leaf Monkey (Presbytis entellus).* Experimental Parasitology, 1997. Vol. 86 (2); p. 155-157.

9. Bhumiratana, A., et al., *Emergence of zoonotic Brugia pahangi parasite in Thailand*. Veterinary World, 2023. Vol. 16 (4); p. 752-765.
10. Lau, Y., et al., *Draft genome of Brugia pahangi: high similarity between B. pahangi and B. malayi.* Parasites and Vectors, 2015. Vol. 8; article 451.
11. National Research Council Committee to Update Science, Medicine, and Animals. Science, Medicine, and Animals. Washington DC: National Academies Press; 2004. Regulation of Animal Research. Available from: https://www.ncbi.nlm.nih.gov/books/NBK24650/
12. Evans, C., et al., *The Domestic Dog as a Laboratory Host for Brugia malayi*. Pathogens, 2022. Vol. 11: 1073.
13. Evans, C., et al., *The Course of Brugia malayi Microfilaremia in Experimentally Infected Cats*. Vector-Borne and Zoonotic Diseases, 2021. 21(8); p. 586-591.
14. (CDC), C.D.C. Cats. 2024; Available from: https://www.cdc.gov/healthy-pets/about/cats.html
15. Ash, L., et al., *Development of Brugia pahangi in the Jird, Meriones unguiculatus, With Notes on Infections in Other Rodents*. Journal of Parasitology, 1970. 56(5); p. 962-968.
16. Ash, L., et al., *Development of Subperiodic Brugia malayi in the Jird, Meriones unguiculatus, With Notes on Infections in Other Rodents*. Journal of Parasitology, 1970. 56(5); p. 969-973.
17. Ah, H., et al., *A Simple Method For Isolation of Brugia pahangi and Brugia malayi Microfilariae.* International Journal of Parasitology, 1974. Vol. 4; p. 677-679.
18. Michalski, M., et al., *The NIH-NIAID Filariasis Research Reagent Resource Center*. PloS Negl Trop Dis, 2011. 5(11): p. e1261.
19. Lok, J.B., et al., *Animal Models for the Study of Immunity in Human Filariasis*. Parasitology Today, 1992. Vol. 8(5); p. 168-171.

20. Malone, J., et al., *Brugia pahangi: Susceptibility and Macroscopic Pathology of Golden Hamsters*. Experimental Parasitology, 1975. Vol. 38; p. 279-290.

21. Suswillo, R., et al., *Infections of Brugia pahangi in Conventional and Nude (Athymic) Mice*. Acta Tropica, 1980. Vol. 37; p. 327-335.

22. Vincent, A., et al., *Life Cycle of Brugia pahangi (Nematoda) in Nude Mice, C3H/HeN (nu/nu)*. Journal of Parasitology, 1982. Vol. 68(4); p. 553-560.

23. Fox, E., et al., *A Comparison of Syngeneic Laboratory Rat-Strains as Hosts for Brugia pahangi.* Transactions of the Royal Society of Tropical Medicine and Hygiene, 1976. Vol. 70(5/6); p. 523.

24. Gusmao, R., et al., *Brugia pahangi: Immunologic Evaluation of the Differential Susceptibility to Filarial Infection in Inbred Lewis Rats*. Experimental Parasitology, 1981. Vol. 52; p. 147-159.

25. Cruickshank, J., et al., *Infection of Inbred and Nude (Athymic) Rats with Brugia spp*. Parasite Immunology, 1983. Vol. 5; p. 527-537.

26. Vincent, et al., *Brugia pahangi: Antibodies and Microfilaremias in Lewis Rats*. Experimental Parasitology, 1983. Vol. 56; p. 381-390.

27. Lawrence, R., *Brugia pahangi in Rats: Increasing the Number of Infective Larvae Inoculated Increases the Percentage of Microfilaremic Rats*. Journal of Parasitology, 1991. Vol. 77(1); p. 173-175.

28. Bell, R., *Brugia pahangi: Quantitative Analysis of Infection in Several Inbred Rat Strains*. Experimental Parasitology, 1999. Vol. 92; p. 120-130.

29. Sanger, I., et al., *Filarial infections of Mastomys natalensis and their relavence for experimental chemotherapy*. Acta Tropica, 1981. Vol. 38; p. 277-288.

30. Mills, B.J., et al., Anthelmintic Laboratory Animal Model for Heartworm, Z.S. LLC, Editor. 2020.
31. Janeway, C.A., et al., *Immunobiology: The Immune System in Health and Disease*. 5 ed. 2001, New York: Garland Science.
32. InformedHealth.org. Cologne, Germany: Institute for Quality and Efficiency in Health Care (IQWiG). The innate and adaptive immune systems. 2023. Available from https://www.ncbi.nlm.nih.gov/books/NBK279396/

# CHAPTER 2

# MATERIALS AND METHODS

## *Animals*

All experiments with adult jirds (*Meriones unguiculatus*; FR3, Oshkosh, Wisconsin), adult male Sprague Dawley rats (*Rattus norvegicus*; Charles River Laboratories, Kingston, New York), and purpose-bred dogs were conducted in accordance with the University of Georgia IACUC Guidelines and approved Animal Use Protocol A2023 03-011.

## *Parasites*

*Brugia pahangi* and *D. immitis* L3 were collected from laboratory-raised *Aedes aegypti* black-eyed Liverpool mosquitoes 15 days after feeding on microfilaremic dog blood (FR3, University of Georgia, Athens, GA). Buckets containing infected mosquitoes were placed in a freezer for one minute and five seconds to immobilize the mosquitoes. Once immobilized, mosquitoes were transferred into a mortar and pestle before being gently crushed. The crushed mosquitoes were then rinsed into a 150-µm mesh sieve contained in a Petri dish with chilled Hanks balanced salt solution (HBSS; MP Biomedicals, Santa Ana, California). This procedure was repeated three times with fresh Petri dishes and chilled HBSS before transferring the sieve to a Petri dish with fresh warm HBSS supplemented with 2% ciprofloxacin (Sigma-Aldrich, St. Louis, MO) to allow larvae to migrate out of the mosquitoes. The sieves were transferred to fresh Petri dishes containing warmed HBSS with 2% ciprofloxacin 3 times. Larvae were isolated from the warm dishes of HBSS with 2% ciprofloxacin into groups of 50 for inoculation.

## *Immune Suppression of Rats*

Immune suppression of adult rats was achieved by following the steroid feeding protocol developed by Zoetis Inc. Rats were fed a dietary admixture of standard laboratory rodent chow and hydrocortisone-21-acetate (Sigma-Aldrich, Saint Louis, MO) at 200 parts per million for 8 days before inoculation and remained on the diet until necropsy at 14 days post infection. The food was prepared by TestDiet® (Richmond, IN) and distributed by Stewart's Feed Service (Lawrenceville, GA).

***Infection of rodents***

Adult jirds were infected by intraperitoneal injection in the right inguinal region with 50 *B. pahangi* L3 (n = 3). Adult male Sprague Dawley rats were infected by intraperitoneal or subcutaneous injection in the right inguinal region with 50 *B. pahangi* (n = 12) or *D. immitis* L3 (n = 3) (Figure 2.1). Six sham-treated rats, three healthy and three immunosuppressed, were injected intraperitoneally in the right inguinal region with approximately 300 µL of Hanks' balanced salt solution (HBSS; MP Biomedicals, Santa Ana, California). Additionally, six rats, three healthy and three immunosuppressed, did not receive a sham injection to determine if immune suppression alone had an impact on cell populations.

***Recovery of larvae***

Jirds and rats were humanely euthanized by carbon dioxide asphyxiation followed by cervical dislocation at 14 days post infection. Intraperitoneal cells and larvae were collected by peritoneal lavage via a 5-mm abdominal incision with approximately 30 mL Dulbecco's Phosphate Buffered Saline (Sigma-Aldrich, SLCP2281) to minimize cell degradation. Recovered larvae were quantified, isolated from the cell suspension, and transferred to 10% neutral-buffered formalin (Epredia, Kalamazoo, MI) for storage. Following peritoneal lavage, all inoculated animals had skins removed and tissues were excised. The skin of each animal was scored using a

blade to disrupt dermal tissue and was allowed to soak in room-temperature Phosphate Buffered Saline (Crystalgen, 221-132-4L) for at least one hour to allow remaining larvae to migrate out. The peritoneal wall, diaphragm, lymphatic tissues, and testicles were soaked in individual Petri dishes in room-temperature PBS for at least one hour as well. The kidneys, liver, spleen, gastrointestinal tract, heart, and lungs were rinsed with PBS. Larvae recovered from soaking or rinsing of tissues were quantified and stored using the previously described method. All excised tissues were stored in 70% ethanol after soaking or rinsing (Figure 2.1).

***Differential cytology***

Following peritoneal lavage and larval collection, cells isolated from the peritoneum of jirds and rats were consolidated into a single 50-mL conical tube per animal. Cells were concentrated by centrifugation to a total volume of 1 mL before being prepared for counting. After vortexing the concentrated cell suspension, a 20 µL sample was removed and added to a 2-mL micro test tube with 20 µL of trypan blue dye (Sigma-Aldrich, T8154-20ML). A cell count was determined by pipetting 10 µL of the dyed sample into the opening of each chamber of a Bio-Rad TC20 counting slide (Bio-Rad, 1450015) and inserting the slide into a Bio-Rad TC20 Automated Cell Counter (Bio-Rad, 1450102). Upon completion of the cell count, a 500 µL sample from each 50-mL conical tube was loaded into a prepared cytospin funnel for centrifugation. This procedure results in a microscope slide with a monolayer of cells adhered to the surface that can then be stained and analyzed. The slides were stained using a Wrights stain kit and a differential cell count was conducted (Figure 2.1).

***Data analysis***

Larval recovery data were not normally distributed, and a Kruskal-Wallis test was performed for comparison of L4 recovery between the control group (intraperitoneally

inoculated jirds) and the treatment groups (intraperitoneally and subcutaneously inoculated *B. pahangi* rats and subcutaneously inoculated *D. immitis* rats). Differential cytology data was not normally distributed and a Friedman test was performed for comparison of cell types among all four groups. Statistical analysis were performed using GraphPad Prism version 10 (GraphPad Software, San Diego, CA).

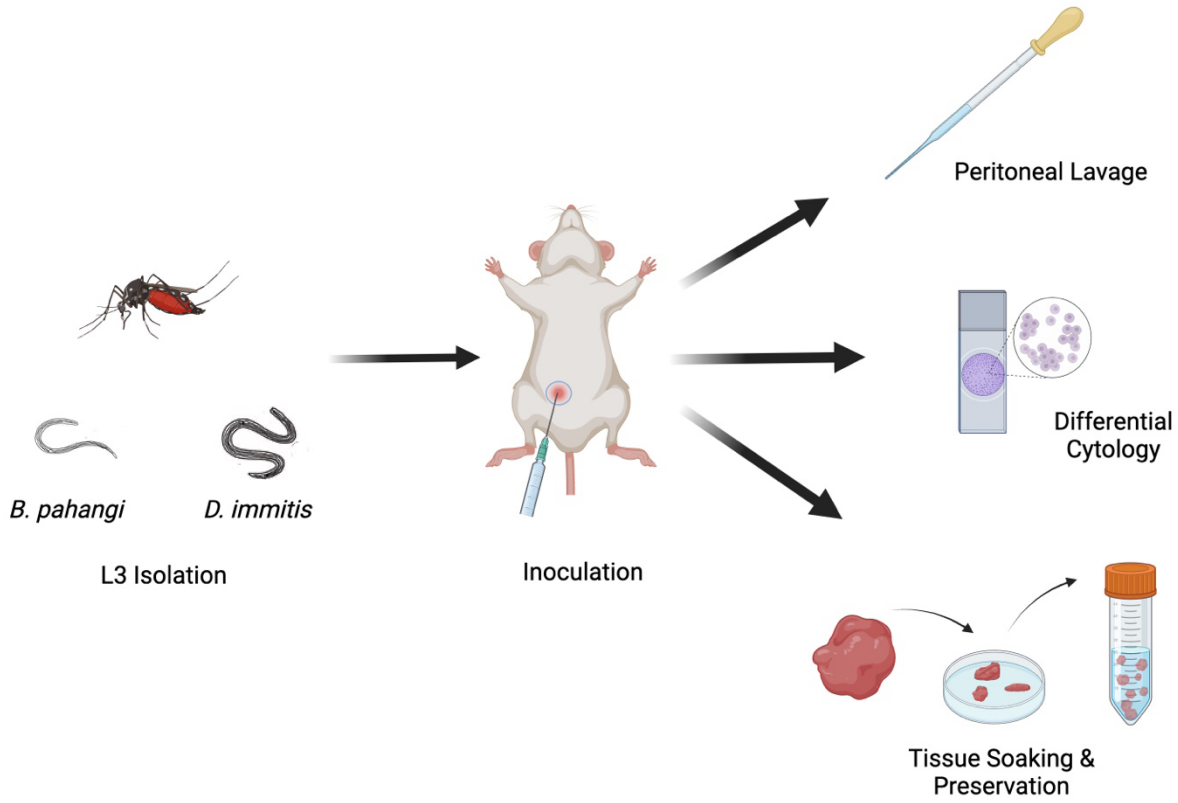


**Figure 2.1**
Experimental design
*Note:* Image created on Biorender.com. *Dirofilaria immitis* and *B. pahangi* L3 were isolated from infected Black-eyed Liverpool strain *Aedes aegypti* mosquitoes 15 days after feeding on microfilaremic dog blood. L3 of each strain were collected into a 1-mL syringe and injected into the inguinal region of each animal either subcutaneously or intraperitoneally, depending on the animal's assigned group. Following inoculation, animals were maintained for 14 days before being humanely euthanized by carbon monoxide asphyxiation followed by cervical dislocation. After euthanasia, a peritoneal lavage was performed to collect any surviving larvae along with peritoneal exudate cells. Larvae were enumerated and saved in PBS. Peritoneal exudate cells were prepared for differential cytology. Additional tissues were harvested from the animal and allowed to soak in PBS for at least one hour to allow any remaining larvae to migrate out before being stored in 70% ethanol.

# CHAPTER 3

# RESULTS

## *Larval Recovery*

In pilot studies, we evaluated larval recoveries from immune-suppressed rats inoculated intraperitoneally with 400 *B. pahangi* L3 each at 7-, 14-, and 90-days post infection (Table 3.1) (Figure 3.1). A mean of 3.4% (SEM = 1.4%) *B. pahangi* L3 were recovered from a total of four inoculated rats while a mean of 32% (SEM = 11.5%) *B. pahangi* L3 were recovered from a total of two jirds used as controls for larval viability at 7-days post infection. At 14-days post infection, a mean of 0.7% (SEM = 0.4) *B. pahangi* L4 were recovered from a total of six inoculated rats while a mean of 15.7% (SEM = 5.5) *B. pahangi* L4 were recovered from a total of three jirds used as larval viability controls. Two rats were maintained until 90-days post infection, but no worms were recovered from either rat while a mean of 23.3% (SEM = 0) adult *B. pahangi* worms were recovered from one control jird.

For the current study, we evaluated larval recoveries from immune-suppressed rats inoculated intraperitoneally or subcutaneously with 50 *B. pahangi* L3 each at 14-days post infection. We also evaluated larval recovery rates from immune-suppressed rats inoculated subcutaneously with 50 *D. immitis* L3 each at 14-days post infection that were intended to serve as controls for the steroid feeding protocol (Table 3.2) (Figure 3.2). A mean of 1.3% (SEM = 0.8 %) *B. pahangi* L4 were recovered from a total of six intraperitoneally inoculated rats and a mean of 0.7% (SEM = 0.7%) *B. pahangi* L4 were recovered from a total of six subcutaneously inoculated rats. A mean of 1.3 % (SEM = 1.3%) *D. immitis* L4 were recovered from a total of

three subcutaneously inoculated control rats. A mean of 33.3% (SEM = 9.4%) *B. pahangi* L4 were recovered from a total of three intraperitoneally inoculated control jirds. A significantly higher number of L4 were recovered from the intraperitoneally inoculated *B. pahangi* jird than from the intraperitoneally inoculated *B. pahangi* rats ($p<0.03$) and from the subcutaneously inoculated *B. pahangi* rats ($p<0.01$).

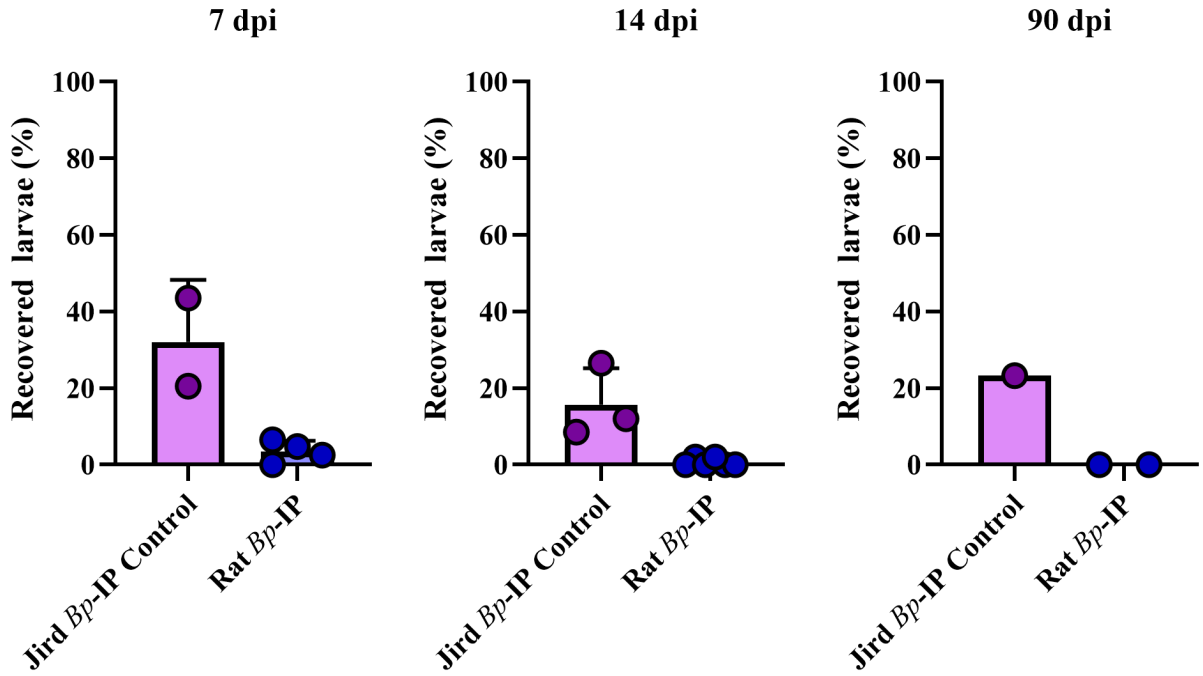


**Figure 3.1**
Larval recovery pilot data of *B. pahangi* L3, L4, and adult worms
Note: Sample sizes for jird *B. pahangi* IP for 7-, 14-, and 90- days post infection were two, three, and one respectively. Sample sizes for rat *B. pahangi* IP for 7-, 14-, and 90- days post infection were four, six, and two respectively.

**Table 3.1**
Larval recovery pilot data of *B. pahangi* L3, L4, and adult worms

| Animal | DPI | Total Larval Recovery | Comments |
|---|---|---|---|
| Jird | 14 | 34 | All alive with no cell attachment |
| Jird | 14 | 106 | All alive with no cell attachment |
| Jird | 14 | 48 | All alive with no cell attachment |
| Jird | 7 | 82 | one larva with cells encasing one half |
| Jird | 7 | 174 | All alive with no cell attachment |
| Jird | 115 (90dpi control) | 93 | All alive with no cell attachment; 40 females, 48 males from lavage; 5 males from testicles; 2.261 x 10^6 mf from lavage |
| Rat | 7 | 10 | 2 live w/ no cell attachment, 1 live w/ minimal cell attachment, 6 with moderate cell attachment, 1 completely encased in cells |
| Rat | 7 | 19 | 4 live w/ no cell attachment, 8 dead with mild/moderate cell attachment, 7 completely encased in cells |
| Rat | 7 | 0 | |
| Rat | 7 | 26 | 5 alive with no cell attachment, 1 alive with minimal cell attachment, 2 dead with no cell attachment, 6 dead with mild/moderate cell attachment, 8 completely encased in cells; 1/4 found in testicles had cell attachment |
| Rat | 14 | 8 | All larvae were dead & had some level of cell attachment |
| Rat | 14 | 0 | |
| Rat | 14 | 0 | |
| Rat | 14 | 0 | |
| Rat | 14 | 0 | |
| Rat | 14 | 8 | All larvae alive, appear to have molted to L4, very small amount of cell attachment on 3/8 |
| Rat | 90 | 0 | |
| Rat | 90 | 0 | |

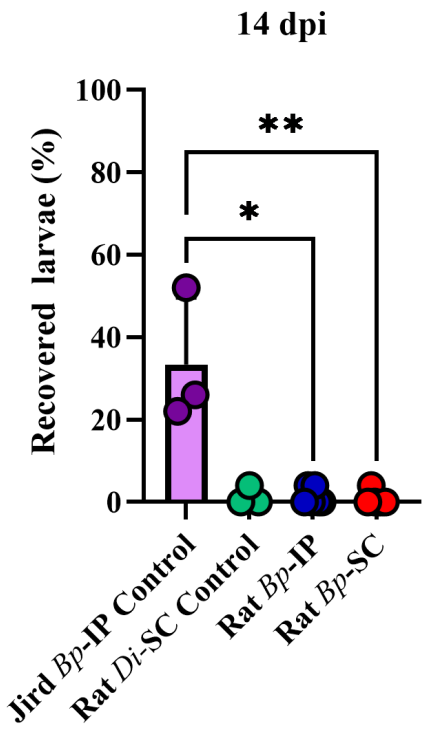


**Figure 3.2**
Total recovery of larvae
Note: Sample sizes for jird *B. pahangi* IP and rat *D. immitis* SC controls were n = 3 each. Sample sizes for rat *B. pahangi* IP and rat *B. pahangi* SC were n = 6 each.

**Table 3.2**
Larval recovery

| ID | Infection type | Animal | Larvae inoculated | # larvae from lavage | # larvae from soakings | Total larvae recovered | % larvae recovered |
|---|---|---|---|---|---|---|---|
| 848 | Bp IP | Jird | 50 | 13 | 0 | 13 | 26 |
| 815 | Bp IP | Rat | 50 | 0 | 0 | 0 | 0 |
| 814 | Bp IP | Rat | 50 | 0 | 0 | 0 | 0 |
| 812 | Bp SQ | Rat | 50 | 0 | 0 | 0 | 0 |
| 813 | Bp SQ | Rat | 50 | 0 | 0 | 0 | 0 |
| 816 | Di SQ | Rat | 50 | 0 | 0 | 0 | 0 |
| 847 | Bp IP | Jird | 50 | 11 | 0 | 11 | 22 |
| 833 | Bp SQ | Rat | 50 | 1 | 1 | 2 | 4 |
| 832 | Bp SQ | Rat | 50 | 0 | 0 | 0 | 0 |
| 834 | Bp IP | Rat | 50 | 1 | 1 | 2 | 4 |
| 835 | Bp IP | Rat | 50 | 0 | 0 | 0 | 0 |
| 836 | Di SQ | Rat | 50 | 0 | 0 | 0 | 0 |
| 858 | Bp IP | Jird | 50 | 26 | 0 | 26 | 52 |
| 827 | Bp IP | Rat | 50 | 0 | 2 | 2 | 4 |
| 826 | Bp IP | Rat | 50 | 0 | 0 | 0 | 0 |
| 824 | Bp SQ | Rat | 50 | 0 | 0 | 0 | 0 |
| 825 | Bp SQ | Rat | 50 | 0 | 0 | 0 | 0 |
| 828 | Di SQ | Rat | 50 | 0 | 2 | 2 | 4 |

*Note:* IP refers to intraperitoneal inoculation; SQ refers to subcutaneous inoculation; Di refers to *D. immitis* infection; Bp refers to *B. pahangi* infection

### *Differential Cell Counts*

Based on the cell attachment noticed in the pilot studies (Table 3.1), we decided to perform differential cell counts on the peritoneal fluid collected from inoculated rats and jirds to identify cells that play a role in the rat immune response to *B. pahangi* or *D. immitis* infection. Four cell types were identified in the differential cytologies: lymphocytes, mast cells, eosinophils, and neutrophils. The only significant difference was in the number of lymphocytes between the intraperitoneally inoculated *B. pahangi* control jirds (mean = 98.8% of all PECs;

SEM = 0.17%) and the steroid-fed sham-treated rats (mean = 85.2 % of all PECs; SEM = 6.5%) ($p<0.0058$) (Figure 3.3).

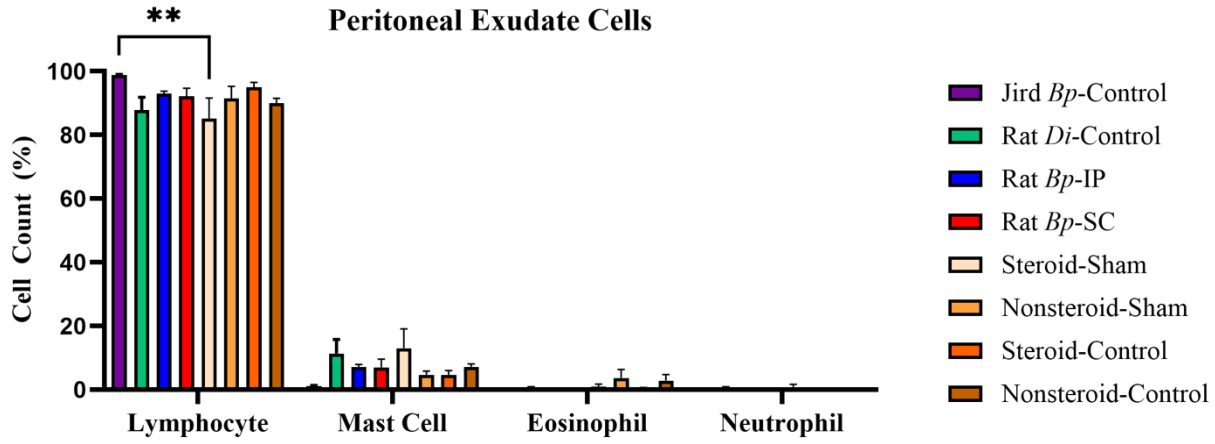


**Figure 3.3**
Peritoneal cell populations of experimentally infected rodents
*Note*: The main takeaway for this figure is that lymphocytes were the primary peritoneal exudate cells identified for all groups. The significance noted between the Jird Bp-Control and Steroid-Sham could likely be attributed to biological variability or small sample sizes. Sample sizes for Rat Bp-IP and Rat Bp-SC were n = 6. All other groups had a sample size of n = 3.

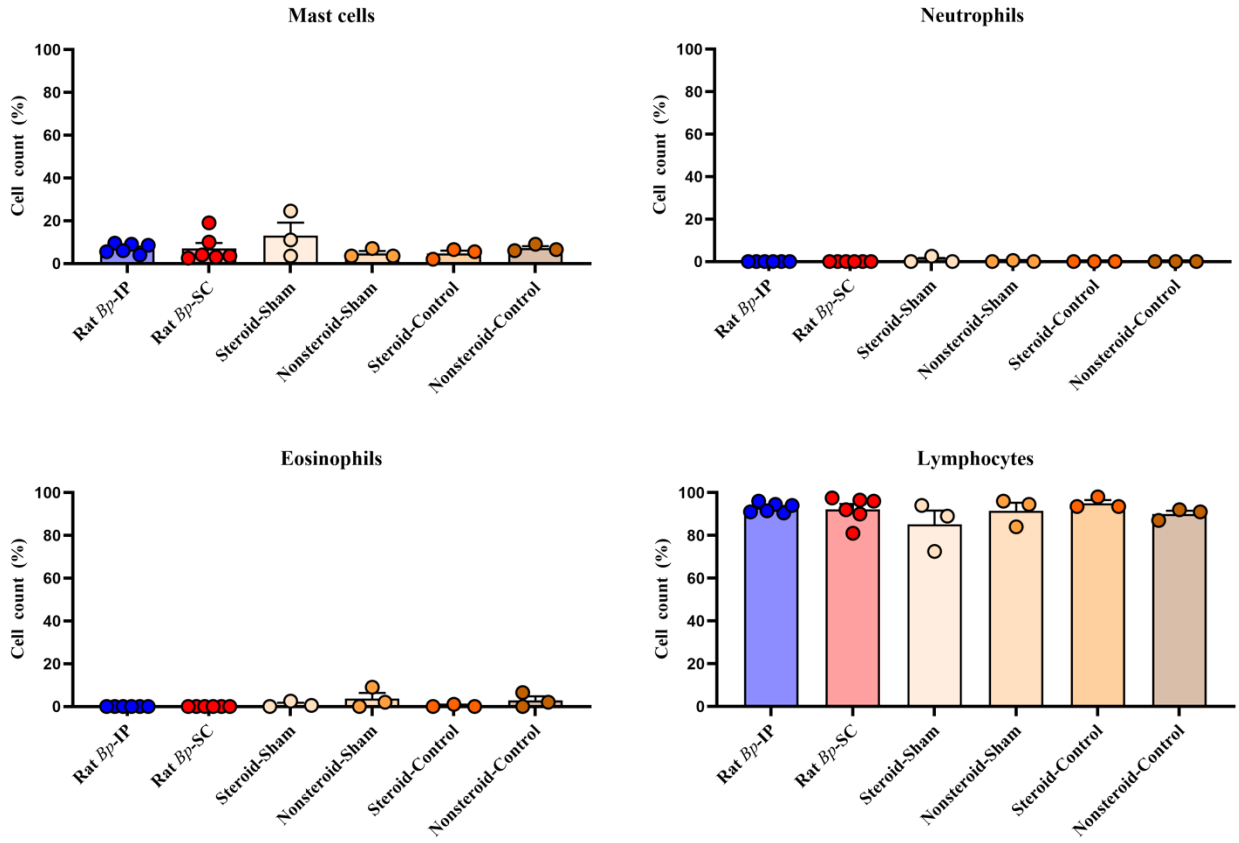


**Figure 3.4**
Individual cell populations of experimentally infected rodents
*Note*: Sample sizes for Rat Bp-IP and Rat Bp-SC were n = 6. All other groups had a sample size of n = 3.

# CHAPTER 4

# DISCUSSION

There is an increasing push for the development of reliable rodent models in filarial parasite research, specifically mouse and rat models. The current model host for production of adult *Brugia* spp. parasites is the Mongolian jird, which is permissive to both subcutaneous and intraperitoneal infections. While the jird model is well established and effective, it does present challenges both from a financial and scientific perspective. I investigated the potential for an immune-suppressed Sprague Dawley rat to serve as a production model for *B. pahangi*. We hypothesized that the immune suppression induced by feeding a dietary mixture containing hydrocortisone-21-acetate, would allow establishment of a *B. pahangi* infection. The data presented here seem to disprove this hypothesis.

The immune suppression protocol used in this study was taken from the hydrocortisone-21-acetate feeding model patented by Zoetis Inc. The Zoetis model was developed as an anthelmintic model for *D. immitis*, canine heartworm, in the Sprague Dawley rat. While *D. immitis* and *B. pahangi* are both classified as filarial parasites, they do not always respond the same to drug exposure or host species. Besides the difference in parasites, another variation between the Zoetis model and this study was that all *D. immitis* infections were subcutaneous while we were interested in intraperitoneal *B. pahangi* infections. We were not able to determine if the reason for lack of establishment of *B. pahangi* in the rat was due to the parasite itself or the route of infection. Inoculating a group of rats intraperitoneally with *D. immitis* may have allowed us to answer this question. This is because the steroid feeding immune suppression method has

been proven successful for subcutaneous *D. immitis* infections but intraperitoneal inoculation has not been attempted. If *D. immitis* L3 inoculated intraperitoneally could survive and mature to adulthood as when inoculated subcutaneously, it would suggest that the lack of establishment of *B. pahangi* in the immune suppressed rat was due to *B. pahangi* itself and not whether the rat is inoculated intraperitoneally or subcutaneously.

As discussed in Chapter 1, several rat and mouse strains show a potential for use as *Brugia* spp. models when inoculated subcutaneously. Though we were primarily interested in establishment of intraperitoneal infections, we did inoculate rats subcutaneously with *B. pahangi* to investigate this possibility as well. While we recovered a small number of L4 from some of these rats, it was significantly fewer than what can be found in the jird and therefore suggests that the Sprague Dawley rat is not as susceptible to subcutaneous infection. We did not maintain these rats long enough for worms to mature to adulthood or produce mf. In the future, this study could be more successful if attempted with a different rat or mouse strain. Athymic rodent strains seem to show the most potential for use as permissive *Brugia* spp. models, after both subcutaneous and intraperitoneal inoculation. As discussed in Chapter 1 of this thesis, it is suspected that establishment of filarial infections could be T-cell dependent. Athymic rodents are a promising model for this reason due to their lack of a thymus and deficiency in T cells.

We did inoculate rats subcutaneously with *D. immitis* L3(n=3) to confirm that our feeding protocol was working as it should; however, we did not recover any larvae at 14 days post infection and did not maintain the rats long enough to recover adult worms. We did not perform *in vitro* cultures to confirm viability of larvae used for infections so low or poor larval viability cannot be ruled out as a cause of the unsuccessful larval recovery. There is also a lack of knowledge about where *D. immitis* larvae migrate between the time of infection and

establishment in the pulmonary artery. The lack of recovery of larvae at 14 days post infection could have been due to a number of reasons. The steroid feeding protocol that we used was adapted directly from the protocol patented by Zoetis, Inc., but was not produced in the same facility. Zoetis produces their hydrocortisone-21-acetate mixture in-house while the food we used was ordered through a vendor. We did not have the food analyzed to confirm it contained the appropriate percentage of steroid. This was due to the limited amount of time we had to complete the study, but feed analysis should be considered in future experiments. We did not have enough *D. immitis* larvae, due to larval production issues, to perform *in vitro* cultures to confirm larval viability before inoculating rats. While we did remove and score the skin and remove and soak internal tissues of the *D. immitis* inoculated rats, there is always a risk that not every larva present will be recovered. This was certainly the case in other larval recovery experiments. For this reason, the tissues were preserved in 70% ethanol for potential PCR analysis in the future. This could help confirm if larvae were present in the skin or tissues and just not recovered.

Four cell types were identified through the differential cell cytologies: neutrophils, eosinophils, mast cells, and lymphocytes. Neutrophils, eosinophils, and mast cells are all involved in the innate immune response. The primary cell type found in all groups was lymphocytes, which are part of the adaptive immune response. Based on our pilot data and the data from this study, worm recovery appeared to decline between 7-days post infection and 14-days post infection, suggesting that the adaptive immune response is primarily responsible for the lack of establishment of *B. pahangi* in the rats. This is also supported by claims made by Cruickshank et al. (1983) that establishment of *B. pahangi* infections in rodents is T lymphocyte dependent. In future studies, flow cytometry could be used to determine if the lymphocytes present are B lymphocytes or T lymphocytes. Flow cytometry was not performed for this study

because we were comparing peritoneal cell populations of rats to those in jirds and unfortunately there are no markers available for use with jirds.

The statistical significance noted in lymphocyte populations between the *B. pahangi* intraperitoneally inoculated control jirds and the steroid-fed, sham-treated rats can likely be attributed to biological variance and small sample sizes. We expect that if larger sample sizes had been used for the control jirds, there would not have been significant differences in cell populations. Based on our results, we believe the large number of lymphocytes found in all groups is due to the adaptive immune response being activated while the other cell types present were either already in the peritoneal cavity or were left over from the innate immune response's attempt to clear the initial infection. The sham-treated and control groups displayed no significant difference in cell populations. For the sham-treated groups, this could be explained by the introduction of foreign material (microscopic mosquito remnants from L3 collection) to the intraperitoneal cavity.

The primary limitation of this study was low mosquito survival and L3 yields from our mosquito colony. We originally intended to inoculate each rat with a total of 100 L3 of either *D. immitis* or *B. pahangi* but were only able to isolate enough L3 to use 50 per inoculation. As mentioned in Chapter 1, a higher dose of inoculum can result in a higher larval recovery. We also intended to have the following four groups: intraperitoneal *B. pahangi*, subcutaneous *B. pahangi*, intraperitoneal *D. immitis*, and subcutaneous *D. immitis* with each group having a total of six animals. However, due to the low mosquito survival and L3 isolation numbers, we had to remove the intraperitoneal *D. immitis* group entirely and had to decrease the number of subcutaneous *D. immitis* animals from 6 to 3.

This study was met with many challenges that likely impacted the reliability of these results. There is much latitude for refinement and adjustment to the study protocol and experimental design that may result in a more successful experiment overall. Other rodent strains, namely athymic rats or mice, should be explored as *Brugia* models. Methods of immune suppression other than the Zoetis steroid feeding protocol could be investigated. If this is the preferred method, subcutaneously inoculated *D. immitis* rats should be used as a control and maintained for at least 120 days, allowing inoculated larvae to reach adulthood and be collected from the pulmonary artery, confirming successful immune suppression. A larger number of L3 should be used for inoculations to allow for better recovery of worms at the time of euthanasia. Despite the lack of successful establishment of infections, there is a wealth of knowledge about the biology and immune functioning of the Sprague Dawley rat. With that in mind, the Sprague Dawley rat could provide a method of studying the immune response to filarial infections that is not yet possible to explore with the Mongolian jird.

# CHAPTER 5

# CONCLUSION

Current large-scale production of *B. pahangi* parasites for research purposes requires the use of the Mongolian jird as a permissive host. This study aimed to evaluate the potential for the immune suppressed Sprague Dawley rat to replace the Mongolian jird as a *B. pahangi* production model. The Sprague Dawley rat is a much more widely used animal model in the scientific research community than the jird, meaning there is a lot more known about the rat's biology and immune functioning. The development of a reliable rat model for *Brugia* spp. would open the door to a range of possibilities for studying the host-parasite interaction and immune response to filarial infection. The rat is also a non-USDA covered species and is therefore not subject to the additional rules and regulations that come with conducting research using USDA covered animals. Finally, the rat is significantly less expensive than the jird, making it a more cost-effective option from a business standpoint. The results of this study indicate that the Sprague Dawley rat as a production model for *B. pahangi* is an unlikely possibility, but a more robust experiment would need to be conducted before this conclusion can be reached definitively.