Population genetic diversity in *Spirocerca lupi*

by

Janishtha Ramesh Mitha

Submitted in partial fulfilment of the requirements for the degree

Magister Scientiae in Genetics

In the faculty of Natural and Agricultural Sciences
University of Pretoria
Pretoria

June 2014

The financial assistance of the National Research Foundation (NRF) towards this research is hereby acknowledged. Opinions expressed and conclusions arrived at, are those of the author and are not necessarily to be attributed to the NRF.

Supervisor: Dr. Pamela J de Waal

Co-supervisors: Prof. Jaco M Greeff and Ms. Kerry Reid

To my dearest grandmother

A woman of great character, overflowing with wisdom and virtue,
the one who taught me life's greatest lessons.

Ganga Jamnadas Rama
16.12.1924 – 18.12.2013
Forever in my heart

ACKNOWLEDGEMENTS

I would sincerely like to thank the all-knowing, mighty One for giving me an amazing support system of people that have made this journey possible. I only mention a few of you here, but there are many more who have shared their wisdom, listened to my long list of excuses and yet still supported and encouraged me.

My brilliant team of supervisors, Pam, Jaco and Kerry, each provided a multitude of different perspectives and opinions that enriched the quality of my work. Pam, thank you so much for your patience and understanding and for your consoling, especially when the tears were flowing. Jaco, thank you for always checking up on me and for always making me see things differently. Kerry, whenever I felt like things were just completely dull, you always managed to somehow make me feel enthusiastic again! Paulette (head of the Genetics department), thank you for your words of encouragement when it seemed like all hope was lost.

Thank you to all in the 'Evolution of Interactions' group! Wiekolize, for joining me on some of my sampling trips which were always full of surprises, Meike, for sharing my low and high moments by supporting me with lots of love (and hugs and back-rubs). Christoff and Zee, you were always willing to share some expert advice. Gayle, Ilkser and Ilkadim, thank you for the amazing friendship throughout these years and for always coming to check up on me and make sure that we get through things together. Carel, thank you for the many laughs you provided in the office, for daily updates on how things were going, interesting conversations about life and for always approving of my baking. Kershney, thank you for your words of motivation which always brightened up my day. Thank you Hester and Ina for caring so much and for being with me on those days when I was nearly passing out. To my friend, Pranesh, a special mention to you for all the help with the printing and your patience with all my last minute requests.

To my family, thank you for being my strength. To my amazing mum, thank you for having the patience to understand that my "tomorrow", "next week" and "by the end of this month", would definitely come someday – you have really endured a lot and I appreciate you for being there! Sharmilmama, your support throughout my life has enabled me to reach where I am today – I truly value all that you have done for me, so selflessly – thank you. Anilmasa (Kanjee), thank you for your much-valued input on my work; even the smallest contribution made the biggest difference. Kishmasa, thank you for your words of wisdom, that I wish every time I could have recorded, and for keeping me healthy and well with your amazing knowledge of the human body. To all my aunts, I can't thank you enough for all the delicious, nutritious meals that were always provided to me so that I would always be well nourished and ready to do great things! To all my cousins, thank you for all the hugs and entertaining times, that kept me smiling throughout the chaos.

Thank you to Anand and my Art of Living Centurion family for supporting me, pointing out my weaknesses and building me up to be a strong individual. Thank you for all the fun times - the dancing, the laughing, the eating and the singing. Thank you to Bibi, for opening up your home and your heart to me, for when I needed a quiet space to be alone and I can never forget the warm hugs I got from Mane that always just made my day! Gurudev, I thank you for the many beautiful gifts you have bestowed upon me, including the wonderful people that surround me every moment of my life, and for the knowledge that this life, is pure celebration! Thanks also to a special little one, who allowed me to grow on a beautiful path, to expand myself, to discover that I am truly capable of much much more – what a wonderful world it is indeed!

Finally, thank you to Vijay for constantly being at my side, throughout the most testing times, for joining me on the most unconventional sampling trips that involved lots of strange things, for lending me your shoulder to cry on, for making sacrifices that encouraged me to work, for pushing me to work when I didn't want to anymore and for understanding when I needed a break. Your unwavering faith and consistent support has been phenomenal and very valuable to me. Thank you so much. Special, special thanks to your Mum for making me feel like her own daughter, always – for shouting me when I didn't want to work and for knowing when I just needed time out and sleep and food! Your family has been understanding and supportive and always showered a lot of love and care on me. If not for them, you would also not have been able to be the amazing person you are, so tons of gratitude to them!

Abundant blessings to all those that have shared this journey with me and reminded me always…
to keep smiling.

Inspiration…

"If you can win over your mind, you can win over the whole world."
- Sri Sri Ravi Shankar,
Founder of the Art of Living Foundation, Spiritual leader and Global humanitarian

"There are many realities. There are many versions of what may appear obvious. Whatever appears as the unshakeable truth, its exact opposite may also be true in another context. After all, one's reality is but perception, viewed through various prisms of context."
- Amish Tripathi,
Author: The Immortals of Meluha

"Creation and destruction are the two ends of the same moment. And everything between the creation and the next destruction is the journey of life."
- Amish Tripathi,
Author: The Oath of the Vayuputras

"Great minds discuss ideas. Average minds discuss events. Small minds discuss people."
- Eleanor Roosevelt,
Former First Lady of the United States

"Sometimes it falls upon a generation to be great. You can be that great generation. Let your greatness blossom."
- Nelson Mandela,
Former President of South Africa, Tata – Father of the Nation

"There is nothing like returning to a place that remains unchanged to find the ways in which you yourself have altered."
- Nelson Mandela,
Former President of South Africa, Tata – Father of the Nation

"First they ignore you, then they ridicule you, then they fight you, and then you win."
- Mahatma Gandhi,
Freedom Fighter and Leader

EXECUTIVE SUMMARY

Spirocerca lupi is a nematode that parasitises canid species across the world. Infested hosts show symptoms associated with the disease called spirocercosis. The parasite is known to cause significant damage to its final host and often leads to death. Treatment of the disease has been a challenge for veterinarians for many years since symptoms of the disease only become apparent at advanced stages of the disease, by which time, the available treatment is considered ineffective. Many studies to date have given insight into the characterisation and description of spirocercosis, however very few studies have been conducted on the molecular biology, biochemistry, genetics, epidemiology, ecology and host-parasite interactions. This study utilises molecular tools to perform genetic analyses to better understand the parasite's population structure which will contribute to improved strategies for the treatment, prevention and control of spirocercosis.

By integrating a population genetic approach with molecular marker data, it is possible to decipher the transmission dynamics of a parasite. For this purpose, microsatellite markers were developed using the FIASCO (fast isolation by AFLPs of sequences containing repeats) protocol and 454 pyrosequencing. Nine polymorphic microsatellite loci were developed to conduct population genetic analyses on *S. lupi* nematodes sampled across three geographical locations in South Africa, namely KwaZulu-Natal (Durban), Eastern Cape (Grahamstown) and Gauteng (Tshwane Metropole). Some of these loci proved to be effective in cross-species amplification testing. The loci were also used to provide molecular evidence that *S. lupi* is in fact found in jackal.

Microsatellites proved to be effective markers in detecting subtle levels of population structuring between the three geographical locations, however allelic frequencies indicated that high amounts of gene flow was occurring. High levels of heterozygosity were found in individual hosts as well as between different hosts, suggesting that little or no inbreeding occurs between *S. lupi* nematodes within the final host. This could be due to the life cycle of the parasite. *Spirocerca lupi* uses a dung beetle intermediate host as well as several paratenic hosts, which allows for significant mixing of parasite genotypes before reaching the final host. In this way, the final host acquires a highly diverse genetic mixture of parasites, which influences mating patterns and results in outbreeding.

This is the first study on *S. lupi* that uses co-dominant markers to study genetic variation and epidemiology across a wide geographical range. The implications of this study are that since *S. lupi* populations have high levels of genetic diversity, they have the genetic potential to adapt to changing environmental conditions as well as the ability to withstand possible treatments that are administered to final hosts. This is critical to consider when control mechanisms are implemented aimed to reduce the risk of infestation in canids. Additional research is required on the impact that different life cycle stages of the nematode have in the different hosts and its implication for effective prevention and control of spirocercosis.

DECLARATION

I, Janishtha Ramesh Mitha declare that the dissertation, which I hereby submit for the degree Magister Scientiae at the University of Pretoria, is my own work and has not previously been submitted by me for a degree at this or any other tertiary institution.

_____ _____

Signature Date

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES