UNIVERSITI PUTRA MALAYSIA

PRODUCTION AND EVALUATION OF MONOCLONAL ANTIBODY AGAINST Strongyloides ratti

MAHMUDA ALIYU

FPSK(P) 2017 6
PRODUCTION AND EVALUATION OF MONOCLONAL ANTIBODY AGAINST *Strongyloides ratti*

By

MAHMUDA ALIYU

Thesis submitted to the School of Graduate Studies, Universiti Putra Malaysia, in Fulfilment of the Requirements for the Degree of Doctor of Philosophy

March 2017
COPYRIGHT

All material contained within the thesis, including without limitation text, logos, icons, photographs and all other artwork, is copyright material of Universiti Putra Malaysia unless otherwise stated. Use may be made of any material contained within the thesis for non-commercial purposes from the copyright holder. Commercial use of material may only be made with the express, prior, written permission of Universiti Putra Malaysia.

Copyright © Universiti Putra Malaysia
DEDICATION

This research work is dedicated with all humility and respect to my beloved parents for their love, guidance, support and patience.
PRODUCTION AND EVALUATION OF MONOCLONAL ANTIBODY AGAINST Strongyloides ratti

By

MAHMUDA ALIYU

March 2017

Currently, most of the available serological diagnostic kits for strongyloidiasis are based on the application of the crude antigens of Strongyloides ratti, which are good, but with less sensitivity for diagnosis of active infection. Hence this study is aimed to produce and evaluate monoclonal antibody for detecting soluble parasite antigen in animal sera prior to hyperinfection and disseminated strongyloidiasis. Strongyloides ratti infection model was established and maintained in immunosuppressed Sprague Dawley rats. Saline extract protein from the infective larvae (iL3) of Strongyloides ratti was used to immunise BALB/c mice. The B-lymphocytes from the spleen taken from the immunised mice were fused with myeloma (SP2/0) cells using 50% polyethylene glycol (PEG) for somatic cell hybridization. The hybridomas were cultured in hypoxantine-aminopterin-thymidine (HAT) medium and cloned by limiting dilutions. Supernatants from the growing positive hybrids were screened by indirect ELISA using 96-well plates coated with the saline extract-protein. The ascites fluid induced by intraperitoneal injection of the antibody-secreting hybridoma cells was purified by a MAb IgG purification kit. The purified antibody (MAb) was characterised by western blots and evaluated in capture ELISA for reactivity against the homologous and heterologous antigens of Ascaris suum, Toxocara canis, Ancylostoma caninum and Toxoplasma gondii. An IgG1 MAb that recognises 30 kDa and 34 kDa associated with strongyloidasis and a cross-reaction with a 30 and 34 kDa for toxocariasis were observed. This indicates that more than one epitope is recognised by the MAb, thus, making it valuable for diagnostic purpose. The MAb was recognised by all Strongyloides ratti antigens and Toxocara canis antigens but did not react with other heterologous antigens in both assays. From the results obtained using the saline extract protein antigen concentration standard curve, it is confirmed that the antigen detection limit by sandwich ELISA was 5 ng/mL, which provides sufficient sensitivity for the diagnosis of Strongyloidiasis. All twelve (12)
strongyloidiasis infected rat sera evaluated for circulating antigen using the MAb produced, have shown antigen-positive reactions in sandwich ELISA. Similar results were obtained from Toxocara infected animal sera. This study concluded that the MAb produced was able to detect strongyloidiasis and toxocariasis in animal models and may also be used for serological diagnosis of strongyloidiasis and toxocariasis in human sera.
Abstrak thesis yang dikemukakan kepada Senate Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah Doktor Falsafah

PENGHASILAN DAN PENILAIAN ANTIBODI MONOKLON TERHADAP *Strongyloides ratti*

Oleh

MAHMUDA ALIYU

Mac 2017

Pengerusi : Ngah Zasmy Unyah, PhD
Fakulti : Perubatan dan Sains Kesihatan

Pada masa ini, kit-kit ujian diagnostik serologi untuk strongiloidiasis kebanyakannya adalah berdasarkan kepada aplikasi antigen-antigen kasar dari *Strongyloides ratti*, kit-kit tersebut sememangnya boleh digunakan, walau bagaimanapun, kit-kit tersebut mempunyai sensitiviti yang rendah terhadap jangkitan aktif. Oleh itu, kajian ini adalah untuk menghasilkan dan menilai antibodi monoklon bagi tujuan mengesan antigen parasit terlarut di dalam sera haiwan sebelum berlakunya jangkitan hiper dan strongiloidiasis tersebar. Model jangkitan *Strongyloides ratti* telah berjaya dihidupkan dan dikekalkan di dalam tikus Sprague Dawley. Protein ekstrak salin dari larva infektif (IL3) dari *Strongyloides ratti* telah digunakan untuk mengimunkan mencit BALB/c. Sel-sel B-lymphocyte yang di keluarkan dari limpa mencit yang telah terimun, disatu dengan sel-sel mieloma (SP2/0) bagi tujuan penghibridan sel-sel somatik dengan bantuan polyethylene glycol (PEG) sebanyak 50% dari kepekatan asal. Medium hypoxantine-aminopterin-thymidine (HAT) digunakan untuk pengkulturan sel-sel hibridoma dan diklonkan dengan kaedah pencairan pengehad. Supernatan dari kacukan hibrid positif yang membangun akan di saring dengan ELISA menggunakan plat 96-berperigi yang telah disaluti dengan protein ekstrak salin. Cecair asites yang terhasil dari suntikan intraperitoneum dengan sel-sel hibridoma perembes-antibodi yang telah ditulenkan menggunakan kit penulenan Mab IgG. Antibodi tertulen (MAb) telah dicirikan dengan kaedah blots western dan dinilai menggunakan ELISA tertangkap untuk melihat reaktiviti terhadap antigen-antigen homologus dan heterologus seperti *Ascaris suum*, *Toxocara canis*, *Ancylostoma caninum* dan *Toxoplasma gondii*. IgG1 MAb yang dikenal pasti pada 30 kDa dan 34 kDa adalah berkait rapat dengan strongyloidiasis dan tindak balas silang dengan 30 kDa and 34 kDa juga telah dikenal pasti. Ini menunjukkan bahawa lebih daripada satu epitope dapat dikesan oleh MAb, oleh itu, ini menjadikan ia penting khusus untuk tujuan diagnostik. MAb telah dapat mengesan *Strongyloides ratti* dan
Toxocara canis tetapi tidak dapat bertindak balas dengan antigen heterologus yang lain pada kedua-dua jenis assai. Dari keputusan yang telah diperolehi dengan menggunakan lengkungan kepekatan piawai, ini mengesahkan bahawa had pengesan antigen oleh ELISA berapit adalah pada 5 ng/mL, yang dapat menyediakan kepekaan yang mencukupi bagi tujuan diagnosis strongyloidiasis. Kesemua dua belas (12) sera tikus yang telah dijangkiti strongyloidiasis bagi tujuan mengesan kehadiran antigen berasal Strongyloides ratti telah menunjukkan reaksi antigen-positif di dalam ELISA berapit. Keputusan yang sama juga dapat dilihat pada sera haiwan yang telah dijangkiti dengan T. canis. Kajian ini menyimpulkan bahawa MAb yang telah terhasil dapat mengesan kedua-dua jangkitan, iaitu, strongyloidiasis dan toksokariasis dalam model haiwan pada kajian yang telah dijalankan, ini juga mungkin dapat digunakan untuk diagnosis serologi terhadap strongyloidiasis dan toksokariasis yang menggunakan sera manusia.
ACKNOWLEDGEMENTS

All thanks, glorifications and total submissions are due to Allah alone, the Creator of the universe, the Preserver and Maintainer of all its affairs, the Administrator and Sustainer of all its workings, the Will behind its phenomena and Disposer of all activities to its successful completion. May the choicest peace and blessings of Allah be upon our leader, Muhammad (SAW), the seal of the prophets, illustrious lamp and guide to the righteous path, and upon his house-hold and companions till eternity.

In the foremost, I thank my parents for nurturing and bringing me up in a moral environment and giving me a direction towards learning and attainment of knowledge.

I would like to express my sincere gratitude and appreciations to my main supervisor: Dr. Ngah Zasmy Al/Unyah for his untiring selfless support and contributions towards the actualization of this work, without whom this project would not have been a reality. Also worthy of note is his effort in providing relevant literatures and wonderful guidance. Surely his undeniable assistance has lifted my spirit high and kept my morals high up to the completion of this important task. I forever remain indebted to him for his wonderful care and kind gestures.

I would like to thank and appreciate the marvelous support of my Co-Supervisors; Associate Professor (Dr) Rukman Awang Hamat and Dr. Roslaini Abd Majid for their useful advice, corrections and encouragement towards my successful completion of this course.

I also thank and appreciate the active role and participation of Dr. Faruku Bande and for his brotherly support of many kinds which have contributed to the success of this journey. The advisory role played by Dr. Ibrahim Abubakar Anka who initiated the zeal to pursue this pristine goal is also worth mentioning. Surely, your kind assistance and encouragement made me complete this important task. I forever remain grateful for your kind assistance.

The role and contributions of my friends Dr. Yusuf Yakubu, Dr. Khalid Jameel Khadeem Al-Zikhiry and Kumareswaran Deevanthan with respect to advice, suggestions and encouragement during this research work is well appreciated. The support and assistance of the Parasitology Lab Technologist; Norhanim Kamaruddin and Assistant Science Officer; Siti Farah Musa is also worth of my appreciation. I am highly indebted to you all as no word will quantify my joy and thankfulness to you.
I shall not conclude without appreciating remarkable and inspiring friends/colleagues like, Drs; A. A. Abubakar, Nafi’u Lawal, Dahiru Sani, Bashiru Garba, Sirajo Bello Shittu, Hassan Maina Ibrahim, Abubakar Danmaigoro and several others so numerous to mention for their patience, understanding and moral support despite odd circumstances towards the realization of my aims. May Allah (SWT) bless them abundantly.

This study would have been futile without the understanding of my Institution, The Usmanu Danfodiyo University, Sokoto in granting me a study leave. I remain indebted to my faculty Dean Prof. A.A. Magaji, my Head of Department and colleagues for their invaluable assistance and all forms of kindness. Finally, I appreciate all who have contributed but whose names could not appear here due to limitation of space. May the garden of Allah’s endless bliss be our final abode!
I certify that a Thesis Examination Committee has met on 13 March 2017 to conduct the final examination of Mahmuda Aliyu on his thesis entitled "Production and Evaluation of Monoclonal Antibody Against Strongyloides ratti" in accordance with the Universities and University Colleges Act 1971 and the Constitution of the Universiti Putra Malaysia [P.U.(A) 106] 15 March 1998. The Committee recommends that the student be awarded the Doctor of Philosophy.

Members of the Thesis Examination Committee were as follows:

Mohamad Aris bin Mohd Moklas, PhD
Associate Professor
Faculty of Medicine and Health Science
Universiti Putra Malaysia
(Chairman)

Zamberi bin Sekawi, PhD
Professor
Faculty of Medicine and Health Science
Universiti Putra Malaysia
/Internal Examiner)

Mohd Nasir bin Mohd Desa, PhD
Associate Professor
Faculty of Medicine and Health Science
Universiti Putra Malaysia
(Internal Examiner)

Constantin Constantinoiu, PhD
Senior Lecturer
James Cook University
Australia
(External Examiner)

\hspace{1cm}

NOR AINI AB. SHUKOR, PhD
Professor and Deputy Dean
School of Graduate Studies
Universiti Putra Malaysia

Date: 28 April 2017
This thesis was submitted to the Senate of Universiti Putra Malaysia and has been accepted as fulfilment of the requirement for the degree of Doctor of Philosophy.

The members of the Supervisory Committee were as follows:

Ngah Zasmy Al/Unyah, PhD  
Senior Lecturer  
Faculty of Medicine and Health Science  
Universiti Putra Malaysia  
(Chairman)

Roslaini Abd Majid, PhD  
Senior Lecturer  
Faculty of Medicine and Health Science  
Universiti Putra Malaysia  
(Member)

Rukman Awang Hamat, PhD  
Associate Professor  
Faculty of Medicine and Health Science  
Universiti Putra Malaysia  
(Member)

ROBIAH BINTI YUNUS, PhD  
Professor and Dean  
School of Graduate Studies  
Universiti Putra Malaysia

Date:
Declaration by graduate student

I hereby confirm that:
• this thesis is my original work
• quotations, illustrations and citations have been duly referenced
• The thesis has not been submitted previously or concurrently for any other degree at any institutions
• intellectual property from the thesis and copyright of thesis are fully-owned by Universiti Putra Malaysia, as according to the Universiti Putra Malaysia (Research) Rules 2012;
• written permission must be owned from supervisor and deputy vice – chancellor (Research and innovation) before thesis is published (in the form of written, printed or in electronic form) including books, journals, modules, proceedings, popular writings, seminar papers, manuscripts, posters, reports, lecture notes, learning modules or any other materials as stated in the Universiti Putra Malaysia (Research) Rules 2012;
• there is no plagiarism or data falsification/fabrication in the thesis, and scholarly integrity is upheld as according to the Universiti Putra Malaysia (Graduate Studies) Rules 2003 (Revision 2012-2013) and the Universiti Putra Malaysia (Research) Rules 2012. The thesis has undergone plagiarism detection software.

Signature: _______________________ Date: ________________

Name and Matric No. Mahmuda Aliyu, GS39821
Declaration by Members of Supervisory Committee

This is to confirm that:

- the research conducted and the writing of this thesis was under our supervision;
- supervision responsibilities as stated in the Universiti Putra Malaysia (Graduate Studies) Rules 2003 (Revision 2012-2013) were adhered to.

Signature: __________________________
Name of Chairman of Supervisory Committee: Dr. Ngah Zasmy AL/Unyah

Signature: __________________________
Name of Member of Supervisory Committee: Dr. Roslaini bin Abd Majid

Signature: __________________________
Name of Member of Supervisory Committee: Associate Professor Dr. Rukman Awang Hamat
# TABLE OF CONTENTS

<table>
<thead>
<tr>
<th>ABSTRACT</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>ABSTRAK</td>
<td>iii</td>
</tr>
<tr>
<td>ACKNOWLEDGEMENTS</td>
<td>v</td>
</tr>
<tr>
<td>APPROVAL</td>
<td>vii</td>
</tr>
<tr>
<td>DECLARATION</td>
<td>ix</td>
</tr>
<tr>
<td>LIST OF TABLES</td>
<td>xvi</td>
</tr>
<tr>
<td>LIST OF FIGURES</td>
<td>xvii</td>
</tr>
<tr>
<td>LIST OF ABBREVIATIONS</td>
<td>xx</td>
</tr>
</tbody>
</table>

## CHAPTER

1. **INTRODUCTION**
   1.1 Background of the study  
   1.2 Statement of the problems  
   1.3 Justification of the research  
   1.4 Research hypotheses  
   1.5 General objective  
   1.5.1 Specific objectives  

2. **LITERATURE REVIEW**
   2.1 Strongyloides species  
   2.1.1 Strongyloides eggs  
   2.1.2 Rhabditiform larvae  
   2.1.3 Filariform larvae  
   2.1.4 Parasitic female  
   2.1.5 Free-living adults  
   2.2 Life cycle of strongyloides  
   2.2.1 Indirect, sexual or heterogonic development  
   2.2.2 Direct, asexual or homogonic development  
   2.3 Strongyloides stercoralis infection  
   2.3.1 Hyperinfection syndrome  
   2.3.2 Disseminated disease  
   2.3.2.1 Strongyloides infections in HIV patients  
   2.3.2.2 Strongyloides infections with HTLV-1 Co-infection  
   2.3.2.3 Strongyloides infections in transplant patients  
   2.3.3 Pathology and clinical signs  
   2.3.4 Prevalence of Strongyloides infection  
   2.3.4.1 Prevalence of Strongyloides infection in Malaysia  
   2.4 Diagnosis of strongyloidiasis  
   2.4.1 Parasitological methods  
   2.4.2 Immunological methods  
   2.4.2.1 Antibody detection  


<table>
<thead>
<tr>
<th>Section</th>
<th>Title</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>2.4.2.1</td>
<td>1.1</td>
<td>Immunofluorescence antibody test (IFAT)</td>
</tr>
<tr>
<td>2.4.2.1</td>
<td>1.2</td>
<td>Enzyme-linked immunosorbent assay (ELISA)</td>
</tr>
<tr>
<td>2.4.2.1</td>
<td>1.3</td>
<td>Western immunoblotting (WI)</td>
</tr>
<tr>
<td>2.4.2.1</td>
<td>1.4</td>
<td>Luciferase immunoprecipitation system (LIPS)</td>
</tr>
<tr>
<td>2.4.2.1</td>
<td>1.5</td>
<td>Dipstick assays</td>
</tr>
<tr>
<td>2.4.2.2</td>
<td></td>
<td>Antigen detection</td>
</tr>
<tr>
<td>2.4.2.3</td>
<td></td>
<td>Immune complex detection</td>
</tr>
<tr>
<td>2.4.2.4</td>
<td></td>
<td>Recombinant antigens for immunodiagnosis</td>
</tr>
<tr>
<td>2.4.3</td>
<td></td>
<td>Molecular methods</td>
</tr>
<tr>
<td>2.4.3.1</td>
<td></td>
<td>Conventional PCR</td>
</tr>
<tr>
<td>2.4.3.2</td>
<td></td>
<td>Quantitative PCR (qPCR)</td>
</tr>
<tr>
<td>2.5</td>
<td></td>
<td>Prevention and control of strongyloidiasis</td>
</tr>
<tr>
<td>2.6</td>
<td></td>
<td>Treatment of strongyloidiasis</td>
</tr>
<tr>
<td>2.7</td>
<td></td>
<td>Immune responses to Strongyloides antigens</td>
</tr>
<tr>
<td>2.8</td>
<td></td>
<td>Applications of monoclonal antibodies in biomedicine</td>
</tr>
<tr>
<td>2.8.1</td>
<td></td>
<td>History of monoclonal antibody</td>
</tr>
<tr>
<td>2.8.2</td>
<td></td>
<td>Monoclonal antibody production</td>
</tr>
<tr>
<td>2.8.3</td>
<td></td>
<td>Diagnostic applications of monoclonal antibodies</td>
</tr>
<tr>
<td>2.8.4</td>
<td></td>
<td>Monoclonal antibodies in diagnosis of infectious diseases</td>
</tr>
<tr>
<td>2.9</td>
<td></td>
<td>Summary of literature review</td>
</tr>
</tbody>
</table>

3. MATERIALS AND METHODS

3.1 Ethical approval

3.1.1 Rats collection

3.1.2 Examination of stool samples

3.1.3 Establishment of *Strongyloides ratti* infection model

3.1.4 Maintenance of *Strongyloides ratti* infection

3.1.5 Experimental design and drug administration

3.1.6 Egg and larval count

3.1.6.1 Modified McMaster worm-egg counting technique

3.1.7 Blood collection for preparation of sera and smears

3.1.8 Histopathological analysis of tissues

3.1.8.1 Tissue fixation

3.1.8.2 Paraffin embedding

3.1.8.3 Tissue sectioning

3.1.8.4 Tissue staining procedure

3.1.9 Preparation of infective larvae (iL3) and free-living stages (FLs)
<table>
<thead>
<tr>
<th>Section</th>
<th>Title</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>3.1.10</td>
<td>Recovery of parasitic females from the gastrointestinal intestinal tracts of infected rats</td>
<td>48</td>
</tr>
<tr>
<td>3.2</td>
<td>Genomic DNA extraction from feces of infected rat</td>
<td>49</td>
</tr>
<tr>
<td>3.2.1</td>
<td>Determination of DNA quality and concentration</td>
<td>50</td>
</tr>
<tr>
<td>3.2.2</td>
<td>Detection of 28S rDNA gene</td>
<td>50</td>
</tr>
<tr>
<td>3.2.3</td>
<td>Gel electrophoresis of PCR product</td>
<td>51</td>
</tr>
<tr>
<td>3.2.4</td>
<td>Purification of PCR product</td>
<td>51</td>
</tr>
<tr>
<td>3.2.5</td>
<td>Gene sequencing and alignment</td>
<td>51</td>
</tr>
<tr>
<td>3.3</td>
<td>Preparation of saline extract protein from infective larvae and other stages of <em>Strongyloides ratti</em></td>
<td>52</td>
</tr>
<tr>
<td>3.3.1</td>
<td>Preparation of excretory/secretory (ES) protein from infective larvae of <em>Strongyloides ratti</em></td>
<td>52</td>
</tr>
<tr>
<td>3.3.2</td>
<td>Source and preparation of heterologous antigens</td>
<td>54</td>
</tr>
<tr>
<td>3.3.3</td>
<td>Determination of protein concentration by Bradford assay method</td>
<td>55</td>
</tr>
<tr>
<td>3.3.4</td>
<td>Protein precipitation for SDS PAGE</td>
<td>56</td>
</tr>
<tr>
<td>3.3.5</td>
<td>Protein analysis based on SDS-PAGE</td>
<td>56</td>
</tr>
<tr>
<td>3.3.6</td>
<td>Staining of polyacrylamide gels</td>
<td>56</td>
</tr>
<tr>
<td>3.3.7</td>
<td>Antigen preparation for immunization</td>
<td>57</td>
</tr>
<tr>
<td>3.3.8</td>
<td>Immunogenicity of the saline extract protein (antigen) of <em>Strongyloides ratti</em></td>
<td>58</td>
</tr>
<tr>
<td>3.4</td>
<td>Culture of parental myeloma (SP2/0) cell line</td>
<td>59</td>
</tr>
<tr>
<td>3.4.1</td>
<td>Sub-culture of myeloma (SP2/0) cells</td>
<td>59</td>
</tr>
<tr>
<td>3.4.2</td>
<td>Cryo-preservation and cryo-recovery of myeloma (SP2/0) cells</td>
<td>60</td>
</tr>
<tr>
<td>3.4.3</td>
<td>Preparation of myeloma (SP2/0) cells for fusion</td>
<td>60</td>
</tr>
<tr>
<td>3.4.4</td>
<td>Preparation of spleen cells for fusion</td>
<td>61</td>
</tr>
<tr>
<td>3.4.5</td>
<td>Cell fusion/hybridoma production</td>
<td>61</td>
</tr>
<tr>
<td>3.4.6</td>
<td>Selection and cloning of hybridomas</td>
<td>62</td>
</tr>
<tr>
<td>3.4.7</td>
<td>Screening for positive hybridoma clones</td>
<td>64</td>
</tr>
<tr>
<td>3.4.8</td>
<td>Preparation of cloning/expansion medium</td>
<td>64</td>
</tr>
<tr>
<td>3.4.9</td>
<td>Sub-cloning of hybridomas</td>
<td>65</td>
</tr>
<tr>
<td>3.4.10</td>
<td>ClonaCell-HY cloning kit products</td>
<td>65</td>
</tr>
<tr>
<td>3.5</td>
<td>Production of ascites containing monoclonal antibody</td>
<td>66</td>
</tr>
<tr>
<td>3.5.1</td>
<td>Monoclonal antibody purification from ascites fluid</td>
<td>67</td>
</tr>
<tr>
<td>3.5.2</td>
<td>Monoclonal antibody isotyping</td>
<td>67</td>
</tr>
<tr>
<td>3.5.3</td>
<td>Monoclonal antibody characterization using western immunoblotting</td>
<td>68</td>
</tr>
<tr>
<td>3.5.4</td>
<td>Quantitative determination of antibodies in sera of infected rats and puppies</td>
<td>68</td>
</tr>
<tr>
<td>3.5.5</td>
<td>Production of rabbit anti-SE polyclonal antibodies</td>
<td>67</td>
</tr>
<tr>
<td>3.5.6</td>
<td>Monoclonal antibody evaluation in sandwich ELISA for detecting <em>Strongyloides ratti</em> and other antigens</td>
<td>67</td>
</tr>
</tbody>
</table>
3.5.7 Statistical analysis 68
3.5.8 Flow chart of methodology 68

4. RESULTS 69
4.1 In vivo maintenance of Strongyloides ratti infection in rats 69
  4.1.1 Chronic Strongyloides infection in a rat model 69
  4.1.2 Effect of corticosteroids on strongyloidiasis 70
  4.1.3 Re-infection of corticosteroid-treated rats with infective larvae of Strongyloides ratti 73
  4.1.4 Histopathology of Strongyloidiasis 75
4.2. Morphological characteristics of the different stages of Strongyloides ratti 75
  4.2.1 Eggs of Strongyloides ratti 76
  4.2.2 Rhabditiform larva of Strongyloides ratti 76
  4.2.3 Filariform larva of Strongyloides ratti 77
  4.2.4 Free-living adults Strongyloides ratti 79
    4.2.4.1 Free-living female Strongyloides ratti 79
    4.2.4.2 Free-living male Strongyloides ratti 79
4.3 Molecular characterization of Strongyloides ratti 80
4.4 SDS-PAGE profile of Strongyloides ratti saline extract proteins 82
    4.4.1 Antibody recognition of the saline extract protein of Strongyloides ratti 83
4.5 Production of monoclonal antibody against Strongyloides ratti (iL3) saline extract protein antigen 84
  4.5.1 Isotyping of MAB-P38-C5 85
  4.5.2 Characterisation of MAb-P38-C5 in western blot 85
  4.5.3 Cross-reactivity testing of MAb-P38-C5 against heterologous antigens in western blot 86
  4.5.4 Evaluation of MAb-P38-C5 in sandwich ELISA for sensitivity 87
    4.5.4.1 Determination of minimum antigen detection limit of MAb-P38-C5 in capture ELISA 87
    4.5.4.2 Cross-reactivity testing of MAb-P38-C5 against heterologous antigens using sandwich ELISA 88
    4.5.4.3 Antigen detection assay from antibody positive sera of strongyloidiasis and toxocariasis 89

5. DISCUSSION 91
5.1 Establishment and maintenance of S. ratti infection model 91
5.2 Hyperinfection and dissemination of infective larvae in tissues 92
5.3 Modified fecal culture technique and morphological characteristics of S. ratti 94
5.4 Molecular confirmation of *S. rattii* 95
5.5 Immunogenicity of the saline extract protein antigen 97
5.6 Monoclonal antibody production and isotyping 98
5.7 Reactivity of the MAb against SDS-PAGE separated antigens in western immunoblotting 100
5.8 Evaluation of the MAb in capture ELISA 102

6. SUMMARY CONCLUSION AND RECOMMENDATIONS FOR FUTURE RESEARCH 104

REFERENCES 107
APPENDICES 136
BIODATA OF STUDENT 158
LIST OF PUBLICATIONS 159
## LIST OF TABLES

<table>
<thead>
<tr>
<th>Table</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>2.1</td>
<td>17</td>
</tr>
<tr>
<td>2.2</td>
<td>18</td>
</tr>
<tr>
<td>3.1</td>
<td>54</td>
</tr>
<tr>
<td>3.2</td>
<td>55</td>
</tr>
<tr>
<td>3.3</td>
<td>55</td>
</tr>
<tr>
<td>3.4</td>
<td>63</td>
</tr>
<tr>
<td>4.1</td>
<td>88</td>
</tr>
</tbody>
</table>

2.1 Global distribution of *Strongyloides stercoralis*  
2.2 Distribution of *Strongyloides stercoralis* in Malaysia  
3.1 Heterologous antigens used for checking cross-reactivity of the MAb-P38-C5 produced by the hybridoma  
3.2 Resolving gel formulations (12%)  
3.3 Stacking gel formulations (5%)  
3.4 ClonaCell-HY Product Formulations  
4.1 Cross-reactivity of MAb-P38-C5 against heterologous antigens by sandwich ELISA
## LIST OF FIGURES

<table>
<thead>
<tr>
<th>Figure</th>
<th>Description</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>2.1</td>
<td>Egg and rhabditiform larva (left and right) respectively of <em>Strongyloides ratti</em> in fresh feces</td>
<td>6</td>
</tr>
<tr>
<td>2.2</td>
<td>Filariform larva of <em>Strongyloides ratti</em> (left) showing the forked tail on the right plate</td>
<td>6</td>
</tr>
<tr>
<td>2.3</td>
<td>Parasitic female of Strongyloides ratti showing its filariform oesophagus and short tail tip</td>
<td>7</td>
</tr>
<tr>
<td>2.4</td>
<td>Free-living adult female (left) and fixed male (right) respectively of <em>Strongyloides ratti</em></td>
<td>8</td>
</tr>
<tr>
<td>2.5</td>
<td>Life cycle of <em>Strongyloides stercoralis</em></td>
<td>10</td>
</tr>
<tr>
<td>2.6</td>
<td>Steps involved in the production of monoclonal antibodies</td>
<td>38</td>
</tr>
<tr>
<td>4.1.1</td>
<td>Eggs output of chronic infection of rats with <em>S. ratti</em>. Letters a, b, and c explains the significant differences (<em>P</em> &lt; 0.05).</td>
<td>69</td>
</tr>
<tr>
<td>4.1.2</td>
<td>Eggs output of <em>Strongyloides ratti</em> after four weeks treatment with 0.25 mg/kg prednisolone. Letters a, b, and c explains the significant differences (<em>P</em> &lt; 0.05).</td>
<td>70</td>
</tr>
<tr>
<td>4.1.3</td>
<td>Photomicrograph of lung section. A: Diffused hemorrhages; B: Massive inflammatory (eosinophilic) cellular infiltration (Magnification X10).</td>
<td>71</td>
</tr>
<tr>
<td>4.1.4</td>
<td>Photomicrograph of liver section. A: Passive congestion; B: Blood pools around the central veins (Magnification X10).</td>
<td>71</td>
</tr>
<tr>
<td>4.1.5</td>
<td>Eggs output of <em>Strongyloides ratti</em> after four weeks treatment with 0.35 mg/kg prednisolone. Letters a, b, and c explains the significant differences (<em>P</em> &lt; 0.05).</td>
<td>72</td>
</tr>
<tr>
<td>4.1.6</td>
<td>Photomicrograph of lung section from infected and treated rat. A: Infective larva of <em>Strongyloides ratti</em> in the alveolar space; B: Hemorrhages; C: Mild cellular infiltration (Magnification X10).</td>
<td>73</td>
</tr>
<tr>
<td>4.1.7</td>
<td>Eggs output of <em>Strongyloides ratti</em> after re-infection of rats and four weeks treatment with 0.35 mg/kg prednisolone. Letters a, b, and c explains the significant differences (<em>P</em> &lt; 0.05).</td>
<td>74</td>
</tr>
<tr>
<td>4.1.8</td>
<td>Photomicrograph of lung section from re-infected and treated rat. A: Infective larva of <em>Strongyloides ratti</em> in tissue; B: Hemorrhages and mild cellular infiltration (Magnification X10).</td>
<td>74</td>
</tr>
<tr>
<td>4.1.9</td>
<td>Photomicrograph of liver sections from re-infected and treated rat showing infective larva (arrows) of <em>Strongyloides ratti</em> in parietal areas of the liver tissues (Magnification X20).</td>
<td>75</td>
</tr>
<tr>
<td>4.21</td>
<td>Egg-containing larva of <em>Strongyloides ratti</em> from fecal samples of infected rats identified by direct fecal smear method (100X; unstained)</td>
<td>76</td>
</tr>
</tbody>
</table>
4.2.2 Rhabditiform larva of *Strongyloides ratti*. A: Rhabditiform oesophagus; B: Patent intestine; C: Pointed tail tip (40X, stained).

4.2.3 a Filariform larva of *Strongyloides ratti* showing its filariform oesophagus (A) that extends to nearly half (½) the total length (20X; stained).

4.2.3 b Filariform larva of *Strongyloides ratti* (100X; stained) showing its characteristic tripartite or notched-tail tip (A) appearance.

4.2.4 Free-living adult female with a rhabditiform eosopahagus (a) and a blunt-end pointed tail tip (b); B: Free-living male *Strongyloides ratti* (40X; stained) showing rhabditiform oesophagus (a) and a blunt-end tail tip which is curved (b) in the male following staining.

4.3.1 DNA purity (A-260/280) and concentration (ng/µL) using Nanodrop spectrophotometer

4.3.2 Agarose gel electrophoresis: PCR amplification of 28S rDNA gene using universal primer; M: 100 bp DNA Ladder; 1 - 6: PCR products from selected samples (180 bp); N: Negative control

4.3.3 Partial sequence results of *Strongyloides ratti* 28S rRNA gene

4.4.1 SDS PAGE profile of Coomassie stained *Strongyloides ratti* antigens: A = Saline extract proteins (iL3); B = Saline extract proteins (Free-living stages); M = Protein ladder (M)

4.4.2 Means of antibody titres of sera of mice immunized with the antigen extract (Mouse 1-3) compared to that of non-immunized mouse serum (NC)

4.4.3 Mouse isotyping strip showing kappa Immunoglobulin (IgG1) sub-class of the monoclonal antibody MAb-P38-C5 purified from ascites fluid

4.4.4 Western Immunoblotting of MAb-P38-C5 against antigens of *Strongyloides ratti*: FL = free-living stagess (saline Extract); PF = Parasitic females (saline extract); ES = Excretory/secretory product from iL3; iL3 = Saline extract from iL3. (30 µg/well protein)

4.4.4.1 Cross reactivity study of MAb-P38-C5 against other parasite antigens. M = Protein ladder; 1 = *Toxocara canis* (SE); 2 = *Toxocara canis* (E/S); 3 = *Ancylostoma caninum*; 4 = *Ascaris suum*; 5 = *Toxoplasma gondii*; 6 = *Strongyloides ratti* (50 µg /well protein)

4.4.5 Relationship between *Strongyloides ratti* antigen (saline extract) concentration and absorbance values showing a cut-off value (Mean absorbance value + 3 standard deviations) from 20 negative rats sera
4.4.6 Antigen levels in 12 *Strongyloides ratti* antibody positive rat sera. NC= negative control serum; PC= positive control serum

4.47 Antigen levels in 6 *Toxocara canis* antibody positive dog sera
### List of Abbreviations

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>ATCC</td>
<td>American Type Culture Collection</td>
</tr>
<tr>
<td>ATL</td>
<td>associated adult t-cell leukemia</td>
</tr>
<tr>
<td>APCs</td>
<td>antigen presenting cells</td>
</tr>
<tr>
<td>Bcc</td>
<td><em>Burkholderia cepacia</em> complex</td>
</tr>
<tr>
<td>BLAST</td>
<td>basic local alignment search tool</td>
</tr>
<tr>
<td>BSA</td>
<td>bovine serum albumin</td>
</tr>
<tr>
<td>CBPP</td>
<td>contagious bovine pleuropneumonia</td>
</tr>
<tr>
<td>CDC</td>
<td>Centre for Disease Control</td>
</tr>
<tr>
<td>CD4</td>
<td>cluster of differentiation 4</td>
</tr>
<tr>
<td>cDNA</td>
<td>complementary de-oxyribonucleic acid</td>
</tr>
<tr>
<td>CE</td>
<td>cystic echinococcosis</td>
</tr>
<tr>
<td>CFA</td>
<td>complete freund’s adjuvant</td>
</tr>
<tr>
<td>CLM</td>
<td>cutaneous larva migrans</td>
</tr>
<tr>
<td>DMEM</td>
<td>dulbecco’s modified eagles medium</td>
</tr>
<tr>
<td>DMSO</td>
<td>dimethyl sulfoxide</td>
</tr>
<tr>
<td>EDTA</td>
<td>ethylene-diamine-tetraacetic acid</td>
</tr>
<tr>
<td>ELISA</td>
<td>enzyme linked immunosorbent assay</td>
</tr>
<tr>
<td>EPG</td>
<td>egg per gram</td>
</tr>
<tr>
<td>ES</td>
<td>excretery/secretory</td>
</tr>
<tr>
<td>FDA</td>
<td>Food and Drug Administration</td>
</tr>
<tr>
<td>FPSK</td>
<td>Faculti Perubatan dan Sains Kesihatan</td>
</tr>
<tr>
<td>HAT</td>
<td>hypoxanthine aminopterine thymidine</td>
</tr>
<tr>
<td>HGPRT</td>
<td>hypoxanthine-guanine-phosphoribosyl-transferase</td>
</tr>
<tr>
<td>HTLV-1</td>
<td>human t-cell lymphotrophic virus 1</td>
</tr>
<tr>
<td>HSCT</td>
<td>hemapoetic stem cell transplant</td>
</tr>
<tr>
<td>HRP</td>
<td>horseradish peroxidase</td>
</tr>
<tr>
<td>IACUC</td>
<td>Institutional Animal Care and Use Committee</td>
</tr>
<tr>
<td>IB</td>
<td>immunoblotting</td>
</tr>
<tr>
<td>IFA</td>
<td>incomplete freund’s adjuvant</td>
</tr>
<tr>
<td>IFAT</td>
<td>immunofluorescent antibody test</td>
</tr>
<tr>
<td>IgA</td>
<td>immunoglobulin a</td>
</tr>
<tr>
<td>IgG</td>
<td>immunoglobulin g</td>
</tr>
<tr>
<td>IgM</td>
<td>immunoglobulin m</td>
</tr>
<tr>
<td>IgE</td>
<td>immunoglobulin e</td>
</tr>
<tr>
<td>IHC</td>
<td>immunohistochemistry</td>
</tr>
<tr>
<td>IL-2</td>
<td>interleukin 2</td>
</tr>
<tr>
<td>IRIS</td>
<td>immune reconstitution inflammatory syndrome</td>
</tr>
<tr>
<td>ITS1</td>
<td>internal transcribed spacer 1</td>
</tr>
<tr>
<td>INF-α</td>
<td>interferon alpha</td>
</tr>
<tr>
<td>INF-γ</td>
<td>interferon gamma</td>
</tr>
<tr>
<td>kDa</td>
<td>kilodalton</td>
</tr>
<tr>
<td>Abbreviation</td>
<td>Full Form</td>
</tr>
<tr>
<td>--------------</td>
<td>-----------</td>
</tr>
<tr>
<td>LAMP</td>
<td>loop-mediated isothermal amplification</td>
</tr>
<tr>
<td>LAT</td>
<td>latex agglutination test</td>
</tr>
<tr>
<td>LDL-C</td>
<td>low-density lipoprotein</td>
</tr>
<tr>
<td>LPS</td>
<td>lipopolysaccharide</td>
</tr>
<tr>
<td>LIPS</td>
<td>luciferase immunoprecipitation system</td>
</tr>
<tr>
<td>MAb</td>
<td>monoclonal antibody</td>
</tr>
<tr>
<td>MAT</td>
<td>modified agglutination test</td>
</tr>
<tr>
<td>MHC</td>
<td>major histocompatibility complex</td>
</tr>
<tr>
<td>MFFT</td>
<td>modified fecal filtration technique</td>
</tr>
<tr>
<td>NaOCl</td>
<td>sodium hypochloride</td>
</tr>
<tr>
<td>NCBI</td>
<td>National Centre for Biotechnology Information</td>
</tr>
<tr>
<td>OD</td>
<td>optical density</td>
</tr>
<tr>
<td>PCR-RFLP</td>
<td>polymerase chain reaction-restriction fragment length polymorphism</td>
</tr>
<tr>
<td>PEG</td>
<td>polyethylene glycol</td>
</tr>
<tr>
<td>PI</td>
<td>post infection</td>
</tr>
<tr>
<td>PVDF</td>
<td>polyvinylidined difluoride</td>
</tr>
<tr>
<td>Psa</td>
<td>pneumococcal surface adhesin</td>
</tr>
<tr>
<td>pH</td>
<td>hydrogen ion concentration</td>
</tr>
<tr>
<td>PBS-T</td>
<td>phosphate buffered saline-tween</td>
</tr>
<tr>
<td>RIA</td>
<td>radio immuno assay</td>
</tr>
<tr>
<td>RIT</td>
<td>radio-immune theraphy</td>
</tr>
<tr>
<td>RNA</td>
<td>ribonucleic acid</td>
</tr>
<tr>
<td>RPM</td>
<td>revolutions per minute</td>
</tr>
<tr>
<td>RPMI</td>
<td>Roswel’s Park Memorial Institute</td>
</tr>
<tr>
<td>rRNA</td>
<td>ribosomal ribonucleic acid</td>
</tr>
<tr>
<td>RT-PCR</td>
<td>reverse transcription polymerase chain reaction</td>
</tr>
<tr>
<td>SDS-PAGE</td>
<td>sodium dodecylsulphate polyacrylamide gel electrophoresis</td>
</tr>
<tr>
<td>STH</td>
<td>soil transmitted helminth</td>
</tr>
<tr>
<td>SE</td>
<td>saline extract</td>
</tr>
<tr>
<td>SSIR</td>
<td>strongyloides stercoralis immunoreactive</td>
</tr>
<tr>
<td>TCS</td>
<td>tissue culture supernatant</td>
</tr>
<tr>
<td>TEMED</td>
<td>tetramethylrhizoinediamine</td>
</tr>
<tr>
<td>Th1</td>
<td>t-helper 1</td>
</tr>
<tr>
<td>Th2</td>
<td>t-helper 2</td>
</tr>
</tbody>
</table>
CHAPTER 1

INTRODUCTION

1.1 Background of the study

Strongyloidiasis is an intestinal parasitic infection of humans that can be manifested as a chronic infection, hyperinfection or a disseminated disease (Ghoshal et al., 2012). The disease has a world-wide distribution but is common and has a prevalence of 30 - 40% of the total population in tropical and sub-tropical countries from African, Asia and South America (Vilela et al., 2009). Immunocompetent individuals usually have asymptomatic infections which are characterised by eosinophilia only (Mir et al., 2006). Human infection is generally acquired through skin penetration by filariform larvae which then develop to gastrointestinal and pulmonary clinical signs (Ardiç, 2009). *Strongyloides stercoralis* is the only human parasitic nematode that is able to persist and replicate within its host (auto-infection cycle) for decades and this lead to infection with high mortality rates especially in immunocompromised hosts (Segarra-Newnham, 2007).

The auto-infection cycle results in a syndrome called hyperinfection which is characterised by intensive invasion and migration of the infective larvae in the lungs and intestines as a result of increase burden due to immunosuppressive conditions in infected patients (Agrawal et al., 2009; Anjos-Ramos et al., 2016). This hyperinfection syndrome results in severe organ pathology and subsequent death due to dissemination of larvae into multiple organs (Lim et al., 2004), but early detection of the infection may alter the fatal course of the disease through proper and timely treatment. Infective larvae may also penetrate the gastrointestinal tract around the colon which can lead to sepsis and multiple organ failure as a result of migration of enteric pathogens along the larval tracts and further symptoms associated with dissemination (Boulware et al., 2007).

Hyperinfection and dissemination are regularly observed in immunocompromised individuals such as patients with Human T-cell Lymphotrophic Viral disease (Carvalho and Da Fonseca Porto, 2004), after organ transplantation (Patel et al., 2008), and has also been reported in patients with Acquired Immunodeficiency Syndrome (Keiser and Nutman, 2004). Patients with autoimmune diseases (Krishnamurthy et al., 2007), congenital immunodeficiencies (Evering and Weiss, 2006), as well as patients under prolonged immunosuppressive therapy for asthma and other inflammatory conditions are high risk individuals (Fardet et al., 2007; Dall'Agnol et al., 2014).
Diagnosis of infections with *Strongyloides stercoralis* is usually done by examination of host’s feces for the presence of eggs/rhabditiform larvae or by coprocultures for the detection of filariform larvae (Knopp *et al*., 2014). Except for hyper-infections and disseminated disease finding, finding eggs in feces has always been very difficult due to the autoinfection cycle. Furthermore, this also affects the number of larvae found during fecal cultures (Habtamu *et al*., 2011). This phenomenon affects the sensitivity of the various conventional methods of diagnoses, thereby leading to negative results even after repeated sample examinations. Molecular detection techniques like PCR have been reported to be both sensitive and specific (Verweij *et al*., 2009) while serological techniques (ELISA, IFA) were reported to be sensitive but with extensive cross reactivity with related nematode parasites (Utzinger *et al*., 2012).

Laboratory diagnosis of Strongyloidiasis can be done using commercially available ELISA kits based on antigens extracted from *S. ratti*. However most of the kits will detect antibodies (Abnova®, Bordier®, AccuDiag™, NovaLisa®, and SCIMEDX etc). The use of crude extract from the filariform larvae of *S. ratti* as antigen in ELISA has always yielded a false positive results (cross reactivity) with sera from patients infected with other helminth parasites (Conway *et al*., 1993; Sultana *et al*., 2012; Ahmad *et al*., 2013). Thus, it is expected that the use of monoclonal antibodies specific to the parasite antigen (protein) would offer high specificity and sensitivity to the serological diagnosis of the disease (Siddiqui and Berk, 2001).

In the absence of early diagnosis of strongyloidiasis, the prognosis of disseminated disease is extremely poor but treatment is usually effective when infections are detected early. It is essential that physicians be aware of the diagnostic shortcomings especially when dealing with immunocompromised patients and the importance of ruling out strongyloidiasis before immunosuppressive regimens should be considered.

*In vivo* maintenance of *Strongyloides stercoralis* has been done in gerbils (*Meriones unguiculatus*) and dogs as a laboratory hosts for research purpose (Shariati *et al*., 2010). However, this can only be achieved if sufficient quantities of infective larvae (iL3) of *Strongyloides stercoralis* are successfully isolated from an infected human patient. This process/technique posses ethical concern and can be tedious and time consuming (Nolan *et al*., 2002). On the other hand, large quantities of infective larvae (iL3) of the rodent species, *Strongyloides ratti* and *Strongyloides venezuelensis* can be easily isolated from naturally infected wild rats (*Rattus* spp) and maintained in rats as laboratory model for research.

Interestingly, *Strongyloides ratti* is a commonly found helminth parasites in wild rodents and it is morphologically and phylogenetically closely related to *Strongyloides stercoralis* which infect humans (Dorris *et al*., 2002; Matsuda *et al*., 2003). Due to these related similarities, an extract from *Strongyloides*
ratti has been used as an antigen in immunodiagnoses of human strongyloidiasis research (Rodrigues et al., 2004, 2007).

1.2 Statement of the problems

Human strongyloidiasis is a potentially life threatening parasitic infection especially in immunocompromised individuals. Diagnosis using conventional methods (direct smear, formol-ether concentration) usually lacks sensitivity due to inconsistent presence of parasite eggs and/or rhabditiform larvae in the fecal samples of majority of patients, even after repeated sample examination from same infected individual (Inês et al., 2011). Invasive methods such as duodenal intubation (Agrawal et al., 2009) and biopsy may be used to collect sample specimens which are then subjected to parasitological methods of diagnosis (Baermann’s technique and culture), and have been reported to be more sensitive than the conventional fecal smearing method. However, the use of these procedures is very unpleasant to patient, which prevents their routine application (Lemos et al., 2003).

Diagnosis by antibody detection (ELISA) has been hampered by lack of specificity as a result of cross-reaction with related helminth parasites. Most available ELISA kits for the diagnosis of strongyloidiasis are based on detection of specific antibodies but not antigen. Thus, they are unable to differentiate active form previously acquired infections and often lead to false positive test results in sero-converted patients. Diagnosis using antigen detection in feces has been reported to be sensitive only in hyperinfection cases but not in chronic infections. Sensitivity and specificity of the serological techniques vary depending on the type of antigen used among many other attributes (Taweethavonsawat et al., 2002). Molecular techniques (PCR) are sensitive and specific but have been reported to be very expensive, time consuming (Levenhagen and Costa-Cruz, 2014), and almost not available in most endemic areas due to poor resource settings. As such, alternative methods have been sought for accurate and rapid diagnosis of Strongyloidiasis.

1.3 Justification of the research

There is no doubt that improved methods for the diagnosis of active strongyloides infection is needed to support clinical diagnosis of the disease and to determine recent prevalence for disease monitoring and control (Segarra-Newnham, 2007). Ideal diagnostic test for strongyloidiasis should have a high sensitivity and specificity for detecting active infection. They should be cheap and applicable for use in the countries where the disease exists (Dekumyoy et al., 2002). This necessitates the continued search for more reliable approaches and robust techniques that would offer high specificity for an accurate and rapid diagnosis of active Strongyloidiasis.
Our approach to diagnosing Strongyloidiasis using monoclonal antibody is based on the assumption that because *S. stercoralis*, being relatively the smallest nematode parasite that are carried through the circulatory system and spend most of its life cycle within the tissues of the infected host, will release some amounts of antigens in the blood of the host individual. Thus, one ought to detect traces of these parasites or its antigen in the blood of the infected host (parasitemia). It is hoped that these monoclonal antibodies can be used to improve the sensitivity/specifcity of capture ELISA for antigen detection as they have been proven effective as a result of their specific-binding ability to target antigens and to overcome the problems associated with the conventional detection assays.

1.4 Research hypotheses

- Saline extract antigen from the infective larvae (iL3) of *Strongyloides ratti* can stimulate in mice an immune response that will be used for the production of monoclonal antibodies specific for strongyloides antigens.
- The monoclonal antibodies specific for antigens of *Strongyloides ratti* can be used to detect strongyloides antigens in sera of infected animals.

1.5 General objective

To develop monoclonal antibodies against *Strongyloides ratti* antigen for use in immunodiagnosis of active Strongyloidiasis in an experimental animal model.

1.5.1 Specific objectives

1. To establish and maintain a *Strongyloides ratti* infection model and to prepare saline extract protein from infective larvae (iL3) for immunisation of mice.
2. To develop and characterize monoclonal antibodies against the saline extract protein of *Strongyloides ratti*.
3. To determine the specificity of the candidate monoclonal antibody (MAbs) against other related parasite antigens.
4. To evaluate the candidate monoclonal antibody in capture ELISA for detecting circulating antigens in sera of *Strongyloides ratti*-infected rats.
REFERENCES


hookworm infection in a co-endemic setting. *PLoS Negl Trop Dis*, 5 (8), e1292.


Concha, R., Harrington, W., & Rogers, A. I. (2005). Intestinal strongyloidiasis: recognition, management, and determinants of


protective immunity to larval *Strongyloides stercoralis* in mice. *Infection and Immunity*, 74, 5730–5738.


Neglected Tropical Diseases, 6 (2), e1522.


fraction of heterologous antigen to detect IgA and IgG in strongyloidiasis using saliva and serum paired samples. *Immunology Letters*, **134** (1), 69–74.


Microbiology, 45 (2), 438–442.


