



**UNIVERSITI PUTRA MALAYSIA**

**MOLECULAR AND IMMUNOLOGICAL IDENTIFICATIONS OF  
*GIARDIA* SP. ISOLATED FROM HUMANS, DOGS AND RODENTS**

**NGAH ZASMY UNYAH**

**FPSK(P) 2004 6**



**MOLECULAR AND IMMUNOLOGICAL IDENTIFICATIONS  
OF *GIARDIA* SP. ISOLATED FROM HUMANS,  
DOGS AND RODENTS**

**By**

**NGAH ZASMY UNYAH**

**Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia  
In Fulfilment of the Requirements for the Degree of Doctor of Philosophy**

**October 2004**



## **DEDICATION**

This work is dedicated to my family and friends.  
Thank you for your support, love and friendship.



Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfilment of the requirements for the degree of Doctor of Philosophy

**MOLECULAR AND IMMUNOLOGICAL IDENTIFICATIONS  
OF *GIARDIA* SP. ISOLATED FROM HUMANS,  
DOGS AND RODENTS**

**By**

**NGAH ZASMY UNYAH**

**October 2004**

**Chairman : Professor Hj. Wan Omar Abdullah, Ph.D.**

**Faculty : Medicine and Health Sciences**

Concentrations and staining methods for identification of *Giardia* parasites in faecal materials from humans and animals are still the routine methods of diagnosis of giardiasis. Introduction of new and sensitive immunological and molecular methods will definitely facilitate diagnosis and the identification of various *Giardia* species that will ultimately improve clinical management and control of disease transmission. The identification of specific proteins of *Giardia* parasites, which are genus and species specific, may further improve the specificity and sensitivity of diagnostic methods. In this study, identification and confirmation of the species of *Giardia* parasite found in Malaysia, particularly in humans, dogs and rodents were done by Polymerase Chain Reaction (PCR) using species-specific primers. Specific Heat-Shock Protein (HSP) as markers for species identification were done using Sodium Dodecyl Sulphate-Polyacrylamide Gel Electrophoresis (SDS-PAGE) and Western Immunoblotting (WI). These markers were used as antigens for the



production of species-specific monoclonal antibodies (MAb). The objectives of this study were: i) to identify the common *Giardia* sp. infecting humans and other mammals (dogs and rodents) in Malaysia using specific *Giardia* primers by PCR; ii) to produce *in vitro* and detect immunogenic HSP using polyclonal sera from rabbit immunised with antigens from known *Giardia* species on WI, and iii) to produce species-specific MAb from the identified HSP markers. Microscopically, this study has observed that *G. intestinalis* (GI) and *G. duodenalis* (GD) were indistinguishable but *G. muris* (GM) can be distinguished from GI or GD. In addition, this study has also confirmed that PCR using species-specific primers was more reliable and accurate in detecting the variant of GI found in humans and dogs. GD isolates recovered from dogs was found to be the actual variant of GI of humans. Clear morphological differentiations and identifications of GM and GI based on microscopical examination were observed and similar results were obtained by PCR using species-specific primers of respective species of *Giardia*. However, the SDS-PAGE and WI failed to identify species-specific HSP markers, but WI using immunised rabbit sera detected four immunogenic HSP, with the molecular weight of 30 kDa, 34 kDa, 58 kDa and 66 kDa. These four immunogenic HSP were detected at 25 °C, 37 °C and 50 °C in both GI and GM. Three species-specific MAbs were produced using the combinations of the four immunogenic HSPs as antigens. These MAbs were designated as (i) [32 kDa HSPMAbGi(IgG3)], (ii) [29 kDa HSPMAbGm(IgM)], and (iii) [20 kDa HSPMAbGi(IgG1)]. [32 kDa HSPMAbGi(IgG3)] MAb was specific for GI variant found in humans, [29 kDa HSPMAbGm(IgM)] MAb was specific for GM and [20 kDa HSPMAbGi(IgG1)] was specific for GI variant in both humans and dogs. These findings suggest that GI is the main causative agent of giardiasis in both humans and urban dogs in Malaysia.



GM is the main *Giardia* parasite infecting rodents in both rural and urban areas in Malaysia.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah Doktor Falsafah

**PENGENALPASTIAN MOLEKUL DAN IMUNOLOGI TERHADAP  
*GIARDIA* SP. YANG DIPENCILKAN DARI MANUSIA,  
ANJING DAN RODEN**

Oleh

**NGAH ZASMY UNYAH**

**Oktober 2004**

**Pengerusi : Profesor Hj. Wan Omar Abdullah, Ph.D.**

**Fakulti : Perubatan dan Sains Kesihatan**

Kaedah pewarnaan dan konsentrasi feses bagi pengenalpastian *Giardia* yang terdapat dalam feses manusia dan haiwan adalah kaedah-kaedah yang masih lagi penting dan digunakan secara rutin dalam diagnosis giardiasis. Kaedah-kaedah terkini imunologi dan molekul dalam diagnosis akan meningkatkan lagi penambahbaikan diagnosis yang akhirnya akan membantu dalam pengurusan klinikal dan kawalan penularan infeksi. Pengenalpastian protein-protein yang spesifik untuk genus and spesies *Giardia* akan dapat meningkatkan lagi kespesifikan pengenalpastian parasit ini. Dalam kajian ini, pengenalpastian spesies *Giardia* yang terdapat di Malaysia, terutamanya yang menjangkiti manusia, anjing dan rodent telah dilakukan melalui kaedah Tindakbalas Berangkai Polimeras (PCR) dengan menggunakan primer yang spesifik untuk spesies *Giardia*. Profil Protein Kejutan Haba (HSP) untuk masing-masing spesies *Giardia* telah dikenalpasti melalui kaedah “Sodium Dodecyl Sulphate–Polyacrylamide Gel Electrophoresis” (SDS-PAGE) dan “Western Immunoblotting” (WI). Tidak ada perbezaan profil proten di antara



ketiga-tiga spesies *Giardia* ini namun terdapat perbezaan antiserum poliklonal arnab yang bertindakbalas secara spesifik dengan HSP untuk masing-masing spesies *Giardia*. HSP juga telah digunakan sebagai antigen bagi penghasilan antibodi monoklonal (MAb) yang spesifik untuk spesies *Giardia*. Objektif kajian ini ialah :

- i) mengenalpasti spesies *Giardia* yang menjangkiti manusia dan haiwan mamalia (anjing dan roden) yang terdapat di Malaysia dengan menggunakan primer yang spesifik bagi spesies untuk *Giardia* dalam kaedah PCR, ii) menghasilkan dan mengesan HSP yang imunogenik sebagai petanda yang spesifik untuk spesies *Giardia* melalui penggunaan serum poliklonal arnab yang telah diimmunisasi dengan antigen spesies *Giardia* yang mana pengenalpastian proten ini adalah melalui kaedah WI, dan iii) menghasilkan MAb yang spesifik untuk spesies *Giardia* menggunakan HSP yang telah dikenalpasti sebagai antigen. Hasil penyelidikan mendapati, kaedah PCR menggunakan primer spesifik untuk spesies dengan tepatnya dapat mengesan dan membezakan di antara varian spesies *G. intestinalis* (GI) yang terdapat dalam manusia dan anjing. Isolat *G. duodenalis* (GD) yang dijumpai pada anjing adalah merupakan salah satu varian GI, sama seperti yang terdapat pada manusia. Perbezaan dan pengenalpastian morfologi dapat dilihat dengan jelas di antara *G. muris* (GM) dan GI, hasil kajian ini adalah sama seperti hasil kajian yang di kesan oleh PCR dengan menggunakan spesifik untuk spesies primer khas untuk GM dan GI. Keputusan penyelidikan ini mendapati, GI adalah agen penyebab utama penyakit giardiasis pada manusia dan anjing terutama di kawasan bandar di Malaysia. GM pula adalah parasit yang menjangkiti khususnya pada roden di kedua-dua kawasan bandar dan luar bandar di Malaysia. Kaedah SDS-PAGE dan WI didapati tidak dapat mengenalpasti kehadiran spesifik untuk spesies protein HSP sebagai petanda, tetapi kaedah WI dengan menggunakan serum arnab



yang telah diimunisasikan, dapat mengenalpasti empat imunogenik protein HSP yang mempunyai berat molekul 30 kDa, 34 kDa, 58 kDa dan 66 kDa. Keempat-empat HSP ini didapati dihasilkan pada suhu 25 °C, 37 °C dan 50 °C oleh GI dan juga GM. Keempat-empat HSP ini merupakan calon yang sesuai digunakan sebagai antigen dalam penghasilan MAb. Tiga MAb yang spesifik kepada spesies *Giardia* telah berjaya dihasilkan dengan menggunakan gabungan keempat-empat jenis imunogenik protein HSP sebagai antigen. Tiga MAb yang telah dihasilkan adalah (i) [32 kDa HSPMAbGi(IgG3)], (ii) [29 kDa HSPMAbGm(IgM)], dan (iii) [20 kDa HSPMAbGi(IgG1)]. MAb [32 kDa HSPMAbGi (IgG3)] didapati spesifik hanya terhadap varian GI yang terdapat pada manusia, MAb [29 kDa HSPMAbGm(IgM)] hanya spesifik kepada GM dan, [20 kDa HSPMAbGi(IgG1)] didapati spesifik kepada kedua-dua varian GI yang terdapat pada manusia dan anjing.



## ACKNOWLEDGEMENTS

I would like to thank all individuals, who have helped me directly or indirectly in this study. My sincere appreciations and thanks goes to my Principal Supervisor and the Head of Medical Parasitology and Entomology Unit, Faculty of Medicine and Health Sciences, UPM, Professor Dr. Hj. Wan Omar Abdullah for his inspiring ideas, guidance, understanding and motivations throughout my study as his graduate student and colleague at the Unit and Co-Supervisor, Prof. Dr. Mak Joon Wah and Dr. Patricia Lim Kim Chooi for their continuing support and guidance; Dr. Lokman Hakim and Dr. Noor Rain Abdullah of the Institute for Medical Research (IMR) for allowing us to use some of the parasite specimens and their facilities; Kuala Lumpur Hospital Diagnostic Laboratory (HKL) for helping in the collection of human faecal specimens; Kuala Lumpur Municipal Council (DBKL) for the assistance in obtaining dogs faecal materials and rodent trapping at Setapak and Chow Kit, Kuala Lumpur.



## TABLE OF CONTENTS

<b>DEDICATION</b>	ii
<b>ABSTRACT</b>	iii
<b>ABSTRAK</b>	vi
<b>ACKNOWLEDGEMENTS</b>	ix
<b>APPROVAL</b>	x
<b>DECLARATION</b>	xii
<b>LIST OF TABLES</b>	xv
<b>LIST OF FIGURES</b>	xv
<b>LIST OF ABBREVIATIONS</b>	xx
<b>CHAPTER</b>	
<b>I INTRODUCTION</b>	1
1.1 General introduction	1
1.2 Hypotheses	2
1.3 Objectives	3
<b>II LITERATURE REVIEW</b>	4
2.1 Morphological identification of <i>Giardia</i>	4
2.2 Identification of <i>Giardia</i> by host specificity	5
2.3 DNA analysis for identification of <i>Giardia</i>	7
2.4 Genetic diversity of <i>G. intestinalis</i>	8
2.5 Giardiasis	9
2.6 Immune responses to <i>Giardia</i> infection	13
2.7 Immunogenicity of <i>Giardia</i> antigens	16
2.8 Diagnosis methods for identification of <i>Giardia</i>	19
<b>III MATERIALS AND METHODS</b>	23
3.1 Materials	23
3.1.1 Materials for isolation and purification of <i>Giardia</i> isolates from faecal materials	24
3.1.2 Materials for <i>in vivo</i> and <i>in vitro</i> maintenance of <i>Giardia</i> isolates	24
3.1.3 Materials for Polymerase Chain Reaction	25
3.1.4 Materials for Sodium Dodecyl Sulphate-Polyacrylamide Gel Electrophoresis and Western Immunoblotting	26
3.1.5 Materials for production of monoclonal antibody	26
3.2 Methods	26
3.2.1 Isolation and purification of <i>Giardia</i> parasites from faecal materials	27
3.2.2 <i>In vivo</i> maintenance of <i>Giardia</i> parasites in gerbils	27
3.2.3 <i>In vitro</i> maintenance of <i>Giardia</i> parasites for the production of Heat-Shock Protein	28
3.2.4 Polymerase Chain Reaction	29
3.2.5 Sodium Dodecyl Sulphate-Polyacrylamide Gel Electrophoresis	30
3.2.6 Western Immunoblotting	31
3.2.7 Production of monoclonal antibody	



## IV RESULTS

- 4.1 Giardia infections in humans, dogs and rodents
- 4.2 Detection and identification of *Giardia* sp. by Polymerase Chain Reaction
  - 4.2.1 Genus identification by PCR using Universal Fungal and Protozoan Primers, GspL and GspR on humans's *Giardia* isolates
  - 4.2.2 Species identification by PCR using species-specific primers for *G. intestinalis*, JW1 and JW2 on humans's *Giardia* isolates
  - 4.2.3 Genus identification by PCR using Universal Fungal and Protozoan Primers, GspL and GspR on dog's *Giardia* isolates
  - 4.2.4 Species identification by PCR using species-specific primers for *G. duodenalis*, JW1 and JW2 on dog's *Giardia* isolates
  - 4.2.5 Genus identification by PCR using Universal Fungal and Protozoan Primers, GspL and GspR on rodent's *Giardia* isolates
  - 4.2.6 Species identification by PCR using species-specific primers for *G. muris*, GMR and GML on rodent's *Giardia* isolates
- 4.3 Differentiation and characterisation of *Giardia* sp. by the production of Heat-Shock Proteins
  - 4.3.1 Sodium Dodesyl Sulphate-Polyacrylamide Gel Electrophoresis on produced Heat-Shock Proteins
- 4.4 Western Immunoblotting on Heat-Shock Proteins produced by Sodium Dodecyl Sulphate-Polyacrylamide Gel Electrophoresis
  - 4.4.1 Western Immunoblotting of polyclonal rabbits sera immunised with *G. intestinalis* antigens (Portland-1, ATCC 30888 and WB, ATCC 30975) against the antigens of *G. intestinalis* isolates from humans
  - 4.4.2 Western Immunoblotting of polyclonal rabbits sera immunised with *G. muris* antigens (IMR 1046) against the antigens of *G. muris* isolates from rodents
  - 4.4.3 Western Immunoblotting of polyclonal rabbits sera immunised with *G. duodenalis* antigens (IMR 2048) against the antigens of *G. duodenalis* isolates from dogs
- 4.5 Production of monoclonal antibody
  - 4.5.1 Production of monoclonal antibody from combination of 30 kDa, 34 kDa, 58 kDa and 66 kDa immunogenic Heat-Shock Proteins antigens of *G. intestinalis*
  - 4.5.2 Production of monoclonal antibody from combination of 30 kDa, 34 kDa, 58 kDa and 66 kDa immunogenic Heat-Shock Proteins antigens of *G. duodenalis*
  - 4.5.3 Production of monoclonal antibody from combination of 30 kDa, 34 kDa, 58 kDa and 66 kDa immunogenic Heat-Shock Proteins antigens of *G. muris*
  - 4.5.4 Identification of *Giardia* isolates from humans using [32 kDaHSPMAbGi(IgG3)], [20 kDaHSPMAbGi(IgG1)] and [29 kDaHSPMAbGm(IgM)] monoclonal antibodies
  - 4.5.5 Identification of *Giardia* isolates from dogs using [32 kDaHSPMAbGi(IgG3)], [20 kDaHSPMAbGi(IgG1)] and [29 kDaHSPMAbGm(IgM)] monoclonal antibodies



4.5.6	Identification of <i>Giardia</i> isolates from rodents using [32 kDaHSPMAbGi(IgG3)], [20 kDaHSPMAbGi(IgG1)] and [29 kDaHSPMAbGm(IgM)] monoclonal antibodies	43
<b>V</b>	<b>DISCUSSION</b>	16
<b>VI</b>	<b>CONCLUSION AND RECOMMENDATIONS</b>	17
	<b>REFERENCES</b>	17
	<b>APPENDICES</b>	22
	<b>BIODATA OF THE AUTHOR</b>	23
	<b>PUBLICATIONS</b>	23



## LIST OF TABLES

Table		Page
1	List of successful <i>in vivo</i> maintenance of <i>Giardia</i> isolates from humans	44
2	List of successful <i>in vivo</i> maintenance of <i>Giardia</i> isolates from dogs	44
3	List of successful <i>in vivo</i> maintenance of <i>Giardia</i> isolates from rodents	45
4	Preparation of TYI-S-33 medium ( <i>In vitro</i> culture media)	223
5	Preparation of Sodium Dodecyl Sulphate-Polyacrylamide Gel Electrophoresis	224
6	Phosphate Buffer Saline, pH 7.2	224
7	Preparation of Western Immunoblotting	225
8	Preparation of 0.85% NaCl	225
9	Methods for Trichrome staining	225
10	Preparation of Polyvinyl Alcohol Fixative	226
11	Giardiasis in humans, dogs and rodents	227



## LIST OF FIGURES

Figure		Page
1	2% agarose gel analysis of PCR for detection of known controls: <i>G. muris</i> (IMR 1046), <i>G. intestinalis</i> (Portland-1, ATCC 30888) and <i>G. duodenalis</i> (IMR 2048).	46
2	2% agarose gel analysis of PCR for detection of <i>Giardia</i> sp. from patients using universal <i>Giardia</i> primers, GspL (5'-AAGTGCGTCAACGAGCAGCT-3') and GspR (5'-TTAGTGCTTTGTGACCATCGA-3') (Mahbubani, <i>et. al.</i> , 1991)	48
3	2% agarose gel analysis of PCR for detection of <i>Giardia</i> sp. from patients using universal <i>Giardia</i> primers, GspL (5'-AAGTGCGTCAACGAGCAGCT-3') and GspR (5'-TTAGTGCTTTGTGACCATCGA-3') (Mahbubani, <i>et. al.</i> , 1991)	49
4	2% agarose gel analysis of PCR for detection of <i>Giardia</i> sp. from dogs using universal <i>Giardia</i> primers, GspL (5'-AAGTGCGTCAACGAGCAGCT-3') and GspR (5'-TTAGTGCTTTGTGACCATCGA-3') (Mahbubani, <i>et. al.</i> , 1991)	50
5	2% agarose gel analysis of PCR for detection of <i>Giardia</i> sp. from dogs using universal <i>Giardia</i> primers, GspL (5'-AAGTGCGTCAACGAGCAGCT-3') and GspR (5'-TTAGTGCTTTGTGACCATCGA-3') (Mahbubani, <i>et. al.</i> , 1991)	51
6	2% agarose gel analysis of PCR for detection of <i>Giardia</i> sp. from gerbils using universal <i>Giardia</i> primers, GspL (5'-AAGTGCGTCAACGAGCAGCT-3') and GspR (5'-TTAGTGCTTTGTGACCATCGA-3') (Mahbubani, <i>et. al.</i> , 1991)	52
7	2% agarose gel analysis of PCR for detection of <i>Giardia</i> sp. from gerbils using universal <i>Giardia</i> primers, GspL (5'-AAGTGCGTCAACGAGCAGCT-3') and GspR (5'-TTAGTGCTTTGTGACCATCGA-3') (Mahbubani, <i>et. al.</i> , 1991)	53
8	2% agarose gel analysis of PCR for detection of <i>Giardia</i> sp. from gerbils using universal <i>Giardia</i> primers, GspL (5'-AAGTGCGTCAACGAGCAGCT-3') and GspR (5'-TTAGTGCTTTGTGACCATCGA-3') (Mahbubani, <i>et. al.</i> , 1991)	54



9	2% agarose gel analysis of PCR for detection of <i>Giardia intestinalis</i> from patients using specific <i>G. intestinalis</i> primers, JW1 (5'-GCGCACCAGGAATGTCTTGT-3') and JW2 (5'-TCACCTACGGATACCTTGTT-3') (Weiss, J.B., <i>et. al.</i> , 1992)	55
10	2% agarose gel analysis of PCR for detection of <i>Giardia intestinalis</i> from patients using specific <i>G. intestinalis</i> primers, JW1 (5'-GCGCACCAGGAATGTCTTGT-3') and JW2 (5'-TCACCTACGGATACCTTGTT-3') (Weiss, J.B., <i>et. al.</i> , 1992)	56
11	2% agarose gel analysis of PCR for detection of <i>Giardia muris</i> from gerbils using specific <i>G. muris</i> primers, GMR (5'-CATAAATCAGTGCAGAGTGTTTC-3') and GML (5'-GAGGAATCATCAGAACCTCGC-3') (Ionas, G., <i>et. al.</i> , 1992)	57
12	2% agarose gel analysis of PCR for detection of <i>Giardia muris</i> from gerbils using specific <i>G. muris</i> primers, GMR (5'-CATAAATCAGTGCAGAGTGTTTC-3') and GML (5'-GAGGAATCATCAGAACCTCGC-3') (Ionas, G., <i>et. al.</i> , 1992)	58
13	2% agarose gel analysis of PCR for detection of <i>Giardia muris</i> from gerbils using specific <i>G. muris</i> primers, GMR (5'-CATAAATCAGTGCAGAGTGTTTC-3') and GML (5'-GAGGAATCATCAGAACCTCGC-3') (Ionas, G., <i>et. al.</i> , 1992)	59
14	2% agarose gel analysis of PCR for detection of <i>Giardia duodenalis</i> from dogs using specific <i>G. intestinalis</i> primers, JW1 (5'-GCGCACCAGGAATGTCTTGT-3') and JW2 (5'-TCACCTACGGATACCTTGTT-3') (Weiss, J.B., <i>et. al.</i> , 1992)	60
15	2% agarose gel analysis of PCR for detection of <i>Giardia duodenalis</i> from dogs using specific <i>G. intestinalis</i> primers, JW1 (5'-GCGCACCAGGAATGTCTTGT-3') and JW2 (5'-TCACCTACGGATACCTTGTT-3') (Weiss, J.B., <i>et. al.</i> , 1992)	61
16	SDS PAGE of heat shock proteins produced by <i>Giardia intestinalis</i> (Portland-1, ATCC 30888 ) at 4°C, 25°C, 37°C, 50°C and 70°C.	62
17	SDS PAGE of heat shock proteins produce by <i>Giardia intestinalis</i> (WB, ATCC 30957) at 4°C, 25°C, 37°C, 50°C and 70°C.	63



18	SDS PAGE of heat shock proteins produced by <i>Giardia muris</i> (IMR 1046) at 4°C, 25°C, 37°C, 50°C and 70°C.	64
19	SDS PAGE of heat shock proteins produced by <i>Giardia duodenalis</i> (IMR 2048) at 4°C, 25°C, 37°C, 50°C and 70°C.	65
20	SDS PAGE of heat shock proteins produced by <i>Giardia</i> sp. Patient No. HKL1 at 4°C, 25°C, 37°C, 50°C and 70°C.	66
21	SDS PAGE of heat shock proteins produced by <i>Giardia</i> sp. Patient No. HKL2 at 4°C, 25°C, 37°C, 50°C and 70°C.	67
22	SDS PAGE of heat shock proteins produced by <i>Giardia</i> sp. Patient No. HKL3 at 4°C, 25°C, 37°C, 50°C and 70°C.	68
23	SDS PAGE of heat shock proteins produced by <i>Giardia</i> sp. Patient No. HKL4 at 4°C, 25°C, 37°C, 50°C and 70°C.	69
24	SDS PAGE of heat shock proteins produced by <i>Giardia</i> sp. Patient No. HKL5 at 4°C, 25°C, 37°C, 50°C and 70°C.	70
25	SDS PAGE of heat shock proteins produced by <i>Giardia</i> sp. Patient No. HKL6 at 4°C, 25°C, 37°C, 50°C and 70°C.	71
26	SDS PAGE of heat shock proteins produced by <i>Giardia</i> sp. Patient No. HKL7 at 4°C, 25°C, 37°C, 50°C and 70°C.	72
27	SDS PAGE of heat shock proteins produced by <i>Giardia</i> sp. Patient No. HKL8 at 4°C, 25°C, 37°C, 50°C and 70°C.	73
28	SDS PAGE of heat shock proteins produced by <i>Giardia</i> sp. Patient No. HKL9 at 4°C, 25°C, 37°C, 50°C and 70°C.	74
29	SDS PAGE of heat shock proteins produced by <i>Giardia</i> sp. Gerbil No. IMR1 at 4°C, 25°C, 37°C, 50°C and 70°C.	75
30	SDS PAGE of heat shock proteins produced by <i>Giardia</i> sp. Gerbil No. IMR2 at 4°C, 25°C, 37°C, 50°C and 70°C.	76
31	SDS PAGE of heat shock proteins produced by <i>Giardia</i> sp. Gerbil No. IMR3 at 4°C, 25°C, 37°C, 50°C and 70°C.	77
32	SDS PAGE of heat shock proteins produced by <i>Giardia</i> sp. Gerbil No. IMR4 at 4°C, 25°C, 37°C, 50°C and 70°C.	78
33	SDS PAGE of heat shock proteins produced by <i>Giardia</i> sp. Gerbil No. IMR5 at 4°C, 25°C, 37°C, 50°C and 70°C.	79



34	SDS PAGE of heat shock proteins produced by <i>Giardia</i> sp. Gerbil No. IMR6 at 4°C, 25°C, 37°C, 50°C and 70°C.	80
35	SDS PAGE of heat shock proteins produced by <i>Giardia</i> sp. Gerbil No. IMR7 at 4°C, 25°C, 37°C, 50°C and 70°C.	81
36	SDS PAGE of heat shock proteins produced by <i>Giardia</i> sp. Gerbil No. IMR8 at 4°C, 25°C, 37°C, 50°C and 70°C.	82
37	SDS PAGE of heat shock proteins produced by <i>Giardia</i> sp. Gerbil No. IMR9 at 4°C, 25°C, 37°C, 50°C and 70°C.	83
38	SDS PAGE of heat shock proteins produced by <i>Giardia</i> sp. Gerbil No. IMR10 at 4°C, 25°C, 37°C, 50°C and 70°C.	84
39	SDS PAGE of heat shock proteins produced by <i>Giardia</i> sp. Gerbil No. IMR11 at 4°C, 25°C, 37°C, 50°C and 70°C.	85
40	SDS PAGE of heat shock proteins produced by <i>Giardia</i> sp. Gerbil No. IMR12 at 4°C, 25°C, 37°C, 50°C and 70°C.	86
41	SDS PAGE of heat shock proteins produced by <i>Giardia</i> sp. Gerbil No. IMR13 at 4°C, 25°C, 37°C, 50°C and 70°C.	87
42	SDS PAGE of heat shock proteins produced by <i>Giardia</i> sp. Gerbil No. IMR14 at 4°C, 25°C, 37°C, 50°C and 70°C.	88
43	SDS PAGE of heat shock proteins produced by <i>Giardia</i> sp. Dog No. DBKL1 at 4°C, 25°C, 37°C, 50°C and 70°C.	89
44	SDS PAGE of heat shock proteins produced by <i>Giardia</i> sp. Dog No. DBKL2 at 4°C, 25°C, 37°C, 50°C and 70°C.	90
45	SDS PAGE of heat shock proteins produced by <i>Giardia</i> sp. Dog No. DBKL3 at 4°C, 25°C, 37°C, 50°C and 70°C.	91
46	SDS PAGE of heat shock proteins produced by <i>Giardia</i> sp. Dog No. DBKL4 at 4°C, 25°C, 37°C, 50°C and 70°C.	92
47	SDS PAGE of heat shock proteins produced by <i>Giardia</i> sp. Dog No. DBKL5 at 4°C, 25°C, 37°C, 50°C and 70°C.	93
48	SDS PAGE of heat shock proteins produced by <i>Giardia</i> sp. Dog No. DBKL6 at 4°C, 25°C, 37°C, 50°C and 70°C.	94
49	SDS PAGE of heat shock proteins produced by <i>Giardia</i> sp. Dog No. DBKL7 at 4°C, 25°C, 37°C, 50°C and 70°C.	95

50	SDS PAGE of heat shock proteins produced by <i>Giardia</i> sp. Dog No. DBKL8 at 4°C, 25°C, 37°C, 50°C and 70°C.	96
51	SDS PAGE of heat shock proteins produced by <i>Giardia</i> sp. Dog No. DBKL9 at 4°C, 25°C, 37°C, 50°C and 70°C.	97
52	SDS PAGE of heat shock proteins produced by <i>Giardia</i> sp. Dog No. DBKL10 at 4°C, 25°C, 37°C, 50°C and 70°C.	98
53	Immunoblotting of heat shock protein antigens produced by <i>Giardia intestinalis</i> (Portland-1, ATCC 30888) at 4°C, 25°C, 37°C, 50°C and 70°C, against rabbit serum immunised with <i>G. intestinalis</i> antigens.	99
54	Immunoblotting of heat shock protein antigens produced by <i>Giardia intestinalis</i> (WB, ATCC 30957) at 4°C, 25°C, 37°C, 50°C and 70°C, against rabbit serum immunised with <i>G. intestinalis</i> antigens.	100
55	Immunoblotting of heat shock proteins antigens produced by <i>Giardia muris</i> (IMR 1046) at 4°C, 25°C, 37°C, 50°C and 70°C., against rabbit serum immunised with <i>G. muris</i> antigens.	101
56	Immunoblotting of heat shock proteins antigens produced by <i>Giardia duodenalis</i> (IMR 2048) at 4°C, 25°C, 37°C, 50°C and 70°C., against rabbit serum immunised with <i>G. duodenalis</i> antigens.	102
57	Immunoblotting of heat shock proteins antigens produced by <i>Giardia</i> sp. Patient No. HKL1 at 4°C, 25°C, 37°C, 50°C and 70°C, against rabbit serum immunised with <i>G. intestinalis</i> antigens.	103
58	Immunoblotting of heat shock proteins antigens produced by <i>Giardia</i> sp. Patient No. HKL2 at 4°C, 25°C, 37°C, 50°C and 70°C, against rabbit serum immunised with <i>G. intestinalis</i> antigens.	104
59	Immunoblotting of heat shock proteins antigens produced by <i>Giardia</i> sp. Patient No. HKL3 at 4°C, 25°C, 37°C, 50°C and 70°C., against rabbit serum immunised with <i>G. intestinalis</i> antigens.	105
60	Immunoblotting of heat shock proteins antigens produced by <i>Giardia</i> sp. Patient No. HKL4 at 4°C, 25°C, 37°C, 50°C and 70°C, against rabbit serum immunised with <i>G. intestinalis</i> antigens.	106
61	Immunoblotting of heat shock proteins antigens produced by <i>Giardia</i> sp. Patient No. HKL5 at 4°C, 25°C, 37°C, 50°C and 70°C, against rabbit serum immunised with <i>G. intestinalis</i> antigens.	107

62	Immunoblotting of heat shock proteins antigens produced by <i>Giardia</i> sp. Patient No. HKL6 at 4°C, 25°C, 37°C, 50°C and 70°C, against rabbit serum immunised with <i>G. intestinalis</i> antigens	108
63	Immunoblotting of heat shock proteins antigens produced by <i>Giardia</i> sp. Patient No. HKL7 at 4°C, 25°C, 37°C, 50°C and 70°C, against rabbit serum immunised with <i>G. intestinalis</i> antigens.	109
64	Immunoblotting of heat shock proteins antigens produced by <i>Giardia</i> sp. Patient No. HKL8 at 4°C, 25°C, 37°C, 50°C and 70°C, against rabbit serum immunised with <i>G. intestinalis</i> antigens.	110
65	Immunoblotting of heat shock proteins antigens produced by <i>Giardia</i> sp. Patient No. HKL9 at 4°C, 25°C, 37°C, 50°C and 70°C, against rabbit serum immunised with <i>G. intestinalis</i> antigens.	111
66	Immunoblotting of heat shock proteins antigens produced by <i>Giardia</i> sp. Gerbil No. IMR1 at 4°C, 25°C, 37°C, 50°C and 70°C, against rabbit serum immunised with <i>G. muris</i> antigens.	112
67	Immunoblotting of heat shock proteins antigens produced by <i>Giardia</i> sp. Gerbil No. IMR2 at 4°C, 25°C, 37°C, 50°C and 70°C, against rabbit serum immunised with <i>G. muris</i> antigens.	113
68	Immunoblotting of heat shock proteins antigens produced by <i>Giardia</i> sp. Gerbil No. IMR3 at 4°C, 25°C, 37°C, 50°C and 70°C, against rabbit serum immunised with <i>G. muris</i> antigens.	114
69	Immunoblotting of heat shock proteins antigens produced by <i>Giardia</i> sp. Gerbil No. IMR4 at 4°C, 25°C, 37°C, 50°C and 70°C, against rabbit serum immunised with <i>G. muris</i> antigens.	115
70	Immunoblotting of heat shock proteins antigens produced by <i>Giardia</i> sp. Gerbil No. IMR5 at 4°C, 25°C, 37°C, 50°C and 70°C, against rabbit serum immunised with <i>G. muris</i> antigens.	116
71	Immunoblotting of heat shock proteins antigens produced by <i>Giardia</i> sp. Gerbil No. IMR6 at 4°C, 25°C, 37°C, 50°C and 70°C, against rabbit serum immunised with <i>G. muris</i> antigens.	117
72	Immunoblotting of heat shock proteins antigens produced by <i>Giardia</i> sp. Gerbil No. IMR7 at 4°C, 25°C, 37°C, 50°C and 70°C, against rabbit serum immunised with <i>G. muris</i> antigens.	118
73	Immunoblotting of heat shock proteins antigens produced by <i>Giardia</i> sp. Gerbil No. IMR8 at 4°C, 25°C, 37°C, 50°C and 70°C, against rabbit serum immunised with <i>G. muris</i> antigens.	119

74	Immunoblotting of heat shock proteins antigens produced by <i>Giardia</i> sp. Gerbil No. IMR9 at 4°C, 25°C, 37°C, 50°C and 70°C, against rabbit serum immunised with <i>G. muris</i> antigens.	120
75	Immunoblotting of heat shock proteins antigens produced by <i>Giardia</i> sp. Gerbil No. IMR10 at 4°C, 25°C, 37°C, 50°C and 70°C, against rabbit serum immunised with <i>G. muris</i> antigens.	121
76	Immunoblotting of heat shock proteins antigens produced by <i>Giardia</i> sp. Gerbil No. IMR11 at 4°C, 25°C, 37°C, 50°C and 70°C, against rabbit serum immunised with <i>G. muris</i> antigens	122
77	Immunoblotting of heat shock proteins antigens produced by <i>Giardia</i> sp. Gerbil No. IMR12 at 4°C, 25°C, 37°C, 50°C and 70°C, against rabbit serum immunised with <i>G. muris</i> antigens.	123
78	Immunoblotting of heat shock proteins antigens produced by <i>Giardia</i> sp. Gerbil No. IMR13 at 4°C, 25°C, 37°C, 50°C and 70°C, against rabbit serum immunised with <i>G. muris</i> antigens.	124
79	Immunoblotting of heat shock proteins antigens produced by <i>Giardia</i> sp. Gerbil No. IMR14 at 4°C, 25°C, 37°C, 50°C and 70°C, against rabbit serum immunised with <i>G. muris</i> antigens.	125
80	Immunoblotting of heat shock proteins antigens produced by <i>Giardia</i> sp. Dog No. DBKL1 at 4°C, 25°C, 37°C, 50°C and 70°C, against rabbit serum immunised with <i>G. duodenalis</i> antigens.	126
81	Immunoblotting of heat shock proteins antigens produced by <i>Giardia</i> sp. Dog No. DBKL2 at 4°C, 25°C, 37°C, 50°C and 70°C, against rabbit serum immunised with <i>G. duodenalis</i> antigens.	127
82	Immunoblotting of heat shock proteins antigens produced by <i>Giardia</i> sp. Dog No. DBKL3 at 4°C, 25°C, 37°C, 50°C and 70°C, against rabbit serum immunised with <i>G. duodenalis</i> antigens.	128
83	Immunoblotting of heat shock proteins antigens produced by <i>Giardia</i> sp. Dog No. DBKL4 at 4°C, 25°C, 37°C, 50°C and 70°C, against rabbit serum immunised with <i>G. duodenalis</i> antigens.	129
84	Immunoblotting of heat shock proteins antigens produced by <i>Giardia</i> sp. Dog No. DBKL5 at 4°C, 25°C, 37°C, 50°C and 70°C, against rabbit serum immunised with <i>G. duodenalis</i> antigens.	130
85	Immunoblotting of heat shock proteins antigens produced by <i>Giardia</i> sp. Dog No. DBKL6 at 4°C, 25°C, 37°C, 50°C and 70°C, against rabbit serum immunised with <i>G. duodenalis</i> antigens.	131

86	Immunoblotting of heat shock proteins antigens produced by <i>Giardia</i> sp. Dog No. DBKL7 at 4°C, 25°C, 37°C, 50°C and 70°C, against rabbit serum immunised with <i>G. duodenalis</i> antigens.	132
87	Immunoblotting of heat shock proteins antigens produced by <i>Giardia</i> sp. Dog No. DBKL8 at 4°C, 25°C, 37°C, 50°C and 70°C, against rabbit serum immunised with <i>G. duodenalis</i> antigens.	133
88	Immunoblotting of heat shock proteins antigens produced by <i>Giardia</i> sp. Dog No. DBKL9 at 4°C, 25°C, 37°C, 50°C and 70°C, against rabbit serum immunised with <i>G. duodenalis</i> antigens.	134
89	Immunoblotting of heat shock proteins antigens produced by <i>Giardia</i> sp. Dog No. DBKL10 at 4°C, 25°C, 37°C, 50°C and 70°C, against rabbit serum immunised with <i>G. duodenalis</i> antigens.	135
90	Immunoblotting of <i>G. muris</i> (IMR 1046), <i>G. duodenalis</i> (IMR2048), <i>G. Intestinalis</i> (Portland-1, ATCC 30888), <i>Cryptosporidium parvum</i> (IMR 11) and <i>Entamoeba histolytica</i> (HK9, ATCC 30015) antigens against 32 kDa HSPMAb <i>G. intestinalis</i> (IgG3).	136
91	Detection of IgG3 monoclonal antibody of 32 kDa HSPMAb <i>G. intestinalis</i> (IgG3).using Iso Typing Detection Kit (GIBCO, USA)	137
92	Immunoblotting of <i>G. muris</i> (IMR 1046), <i>G. duodenalis</i> (IMR2048), <i>G. intestinalis</i> (Portland-1, ATCC 30888), <i>Cryptosporidium parvum</i> (IMR 11) and <i>Entamoeba histolytica</i> (HK9, ATCC 30015) antigens against 29 kDa HSPMAb <i>G. muris</i> (IgM).	138
93	Detection of IgM monoclonal antibody of 29 kDa HSPMAb <i>G. muris</i> (IgM).using Iso Typing Detection Kit (GIBCO, USA)	139
94	Immunoblotting of <i>G. muris</i> (IMR 1046), <i>G. duodenalis</i> (IMR2048), <i>G. intestinalis</i> (Portland-1, ATCC 30888), <i>Cryptosporidium parvum</i> (IMR 11) and <i>Entamoeba histolytica</i> (HK9, ATCC 30015) antigens against 20 kDa HSPMAb <i>G. intestinalis</i> (IgG1).	140
95	Detection of IgG1 monoclonal antibody of 20 kDa HSPMAb <i>G. intestinalis</i> (IgG1).using Iso Typing Detection Kit (GIBCO, USA)	141
96	Immunoblotting of randomly selected antigens of <i>Giardia</i> isolates from patients, gerbils, dogs, <i>Cryptosporidium parvum</i> , (IMR 11), <i>Giardia intestinalis</i> (Portland-1, ATCC 30888), <i>Giardia duodenalis</i> (IMR 2048) and <i>Entamoeba histolytica</i> (HK9, ATCC 30015) against 32 kDa HSPMAb <i>G. intestinalis</i> (IgG3).	142