



UNIVERSITI PUTRA MALAYSIA

**EPIDEMIOLOGY OF LEPTOSPIRAINTERROGANS SEROVAR
HARDJO
INFECTION IN CATTLE**

SITI KHAIRANI BINTI BEJO

IB 2001 3

**EPIDEMIOLOGY OF *LEPTOSPIRA INTERROGANS* SEROVAR *HARDJO*
INFECTION IN CATTLE**

By

SITI KHAIRANI BINTI BEJO

**Thesis Submitted in Fulfilment of the Requirement for the degree of Doctor
of Philosophy in the Institute of Bioscience
Universiti Putra Malaysia**

August 2001



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

EPIDEMIOLOGY OF *LEPTOSPIRA INTERROGANS* SEROVAR *HARDJO* INFECTION IN CATTLE

By

SITI KHAIRANI BINTI BEJO

August 2001

Chairman: Prof. Dr. Abdul Rani Bahaman

Faculty: Institute of Bioscience

The serological prevalence of *Leptospira interrogans* serovar *hardjo* (hereafter referred to serovar *hardjo*) infection in cattle in this present study was 30%. Water samples from stagnant water, pond water, tank water and drain water collected from the farms were positive to *Leptospira biflexa* (40%). Twenty-four per cent of soil samples obtained from three different types of soil namely clay, loam and sand in the farms were also positive to *Leptospira biflexa*. However, serovar *hardjo* or other pathogenic leptospires were not isolated in the urine, soil and water samples collected in the farms. Clinical sign of leptospiral infection was not observed in the cattle on the farms. The leptospiral isolates were further characterized using bacterial restriction endonuclease DNA analysis (BRENDA), polyacrylamide gel electrophoresis (PAGE) and Western blotting. It was confirmed that the leptospiral isolates did not belong to serovar *hardjo*.



Under experimental condition it was demonstrated that cattle are able to maintain serovar *hardjo*. Six female 8-months-old Kedah-Kelantan calves were used in this trial. Leptospiremia occurred as early as 7 days post-inoculation and lasted for 13 days following intra-conjunctival inoculation. Antibody against serovar *hardjo* was first detected at day 7 post inoculation, then raised to a high level at day 14 post-inoculation and maintained at the same level up to 365 days post inoculation. Leptospiruria was first detected on day 49 post inoculation and maintained up to day 147 post-inoculation. Histologically serovar *hardjo* was detected in the renal tubule at the end of the trial using immunoperoxidase staining. No clinical signs of leptospiral infection was observed in the same animals throughout the trial. Identification of the leptospiral isolates obtained from the inoculum and urine samples of the experimental animals using bacterial restriction endonuclease DNA analysis (BRENDA) and polymerase chain reaction (PCR) showed that both isolates were serovar *hardjo*.

The study showed that serovar *hardjo* can survive in rain water up to 264 hours (11 days) under experimental condition. *Leptospira interrogans* serovar *hardjo* can survive up to 72 hours (3days) in diluted urine in Malaysian field condition and up to 984 hours (41 days) at 4°C. *Leptospira interrogans* serovar *hardjo* can survive in chlorinated drinking water up to 120 hours (5 days) but was killed immediately in seawater. The organism can survive in soil samples



up to 144 hours (6 days). The contaminated environment with serovar *hardjo* can transmit infection of the organism to susceptible animals.

It is evident that serovar *hardjo* infection is present in cattle farms in Malaysia. Cattle in Malaysia have a potential of maintaining serovar *hardjo*. *Leptospira interrogans* serovar *hardjo* has been shown to survive in water and soil for a long time in Malaysian field condition and the organisms can be transmitted to susceptible animals.

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

EPIDEMIOLOGI JANGKITAN *LEPTOSPIRA INTERROGANS* SEROVAR
HARDJO PADA LEMBU

Oleh:

SITI KHAIRANI BINTI BEJO

Ogos 2001

Pengerusi: Prof. Dr. Abdul Rani bin Bahaman

Fakulti: Institut Biosains

Prevalan jangkitan *Leptospira interrogans* serovar *hardjo* (selepas daripada ini dipanggil sebagai serovar *hardjo*) pada lembu dalam kajian serologi adalah 30%. Sampel air bertakung, air kolam, air tangki dan air parit yang diambil dari ladang lembu adalah positif kepada *Leptospira biflexa* (40%). Tiga jenis tanah yang berbeza seperti tanah liat, tanah peroi dan tanah pasir telah di ambil dari ladang tersebut dan didapati 24% sampel tanah juga positif kepada *Leptospira biflexa*. Walaubagaimanapun *Leptospira interrogans* serovar *hardjo* dan lain-lain leptospira patogenik tidak dapat diasingkan dari sampel air kencing lembu, tanah dan air yang diambil dari ladang tersebut. Kesemua lembu di ladang kajian tidak ada menunjukkan tanda-tanda klinikal jangkitan *Leptospira*. Isolat *Leptospira* seterusnya dicirikan menggunakan analisa DNA bakteria endonuklias penyekat, elektroporesis gel poliakrilamid dan pemblotan Western. Isolat *Leptospira* yang diasingkan dalam kajian ini adalah pasti bukan serovar *hardjo*.

Dalam keadaan eksperimen menunjukkan lembu dapat penyaraan serovar *hardjo*. Enam ekor lembu betina baka Kedah-Kelantan berumur lapan bulan telah digunakan dalam percubaan ini. Leptospiremia berlaku seawal tujuh hari selepas inokulasi dan berakhir sehingga hari ke-13 berikutan inokulasi-ke dalam konjunktiva. Antibodi terhadap serovar *hardjo* telah dikesan pertama kalinya pada hari ke tujuh selepas inokulasi, kemudian meningkat ke tahap yang tinggi pada hari ke-14 selepas inokulasi dan seterusnya kekal pada tahap yang sama sehingga hari ke-365 selepas inokulasi. Leptospiruria telah dikesan pertama kalinya pada hari ke-49 selepas inokulasi dan kekal sehingga hari ke-147 selepas inokulasi. Kajian histologi menggunakan pewarnaan immunoperoksides mengesan serovar *hardjo* di tubul ginjal pada akhir percubaan. Tanda klinikal jangkitan leptospira tidak ditunjukkan oleh kesemua lembu sepanjang percubaan ini. Pengenalpastian menggunakan kaedah analisa DNA bakteria endonuklias penyekat dan tindakbalas berangkai polimerase menunjukkan kedua-dua isolat dari lembu ujikaji dan inokulum adalah dikenalpasti sebagai serovar *hardjo*.

Dalam keadaan eksperimen serovar *hardjo* boleh terus hidup sehingga 264 jam (11 hari) didalam air hujan. Dalam cuaca persekitaran Malaysia serovar *hardjo* boleh terus hidup sehingga 72 jam (3 hari) di dalam air kencing yang dicairkan. Pada suhu 4°C serovar *hardjo* terus hidup sehingga 984 jam (41 days).

Leptospira interrogans serovar *hardjo* boleh terus hidup dalam air paip yang berklorin sehingga 120 jam (5 hari) tetapi mati serta merta di dalam air laut. Organisma ini dapat terus hidup dalam tanah sehingga 144 jam (6 hari). Alam sekitar yang telah dicemari oleh serovar *hardjo* dapat menyebarkan jangkitan oleh organisma ini kepada haiwan yang mudah terkena jangkitan.

Kesimpulannya, jangkitan serovar *hardjo* adalah terdapat di ladang lembu di Malaysia. Lembu di Malaysia berpotensi untuk menyara serovar *hardjo*. *Leptospira interrogans* serovar *hardjo* boleh terus hidup di air dan tanah di keadaan persekitaran Malaysia dan organisma ini dipercayai boleh disebarkan kepada haiwan yang mudah terkena jangkitan.

AKNOWLEDGEMENTS

I am exceedingly grateful to my supervisor, Professor Dr. Abdul Rani Bahaman for his advice, guidance, patience, simulating discussions and constant encouragement throughout the planning and execution of this study. His sacrifices and perseverance will forever be remembered. Sincere thanks are also due to Dr. Abdul Rahim Mutalib, Associate Prof. Dr. Mohd Zamri Saad and Dr. Nadzri Salim who have provided advice, helpful in discussions and enlightening comments that have improved this study. I would like to express my deep appreciation and gratitude to the followings who have been of great help in my study: En. Hamzah Adam, Dr. Mohd. Shah Abdul Majid, En. Mohd. Noh Manap, En . Jamil Abdul Samad, En. Mustaffa Hj. Dollah, Cik Noorliza Pargini and members of the Institute of Bioscience and Faculty of Veterinary Medicine for sharing their technical skills and their ever available assistances. I also would like to express my special thanks to all staff in the Beef Unit of Universiti Putra Malaysia, Rancangan Daging Penusu Padang Hijau in Johor and MARDI Bukit Redan in Pahang for their assistance in collecting samples.

Finally, I wish to express my deepest appreciation to my late father, late grandmother and my mother, brothers Hair, Khairulnizan and Khairudin and sister Khairunniza.



TABLE OF CONTENTS

ABSTRACT	ii
ABSTRAK	v
ACKNOWLEDGEMENTS	viii
APPROVAL SHEET	ix
DECLARATION SHEET	xi
LIST OF TABLES	xvi
LIST OF FIGURES	xviii
LIST OF ABBREVIATIONS	xxii

CHAPTER

1	INTRODUCTION	1
2	LITERATURE REVIEW	5
	Leptospirosis	5
	Morphology of Leptospire	5
	Classification of Leptospire	6
	Cultural Characteristic of Leptospire	7
	Epidemiology of Leptospirosis	9
	Pathogenesis of Leptospirosis in Cattle	13
	Immune Response to Leptospiral Infection	15
	Clinical Signs of Leptospirosis in Cattle	15
	Laboratory Techniques in the Diagnosis of Leptospiral Infection in Cattle	17
	Dark-field Microscopy	17
	Microbiological Culture	18
	Microscopic Agglutination Test (MAT)	19
	Enzyme-link Immunosorbent Assay (ELISA)	21
	Electron Microscopy	23
	Histological Staining Technique	23
	Polyacrylamide Gel Electrophoresis (PAGE)	24
	Bacterial Restriction Endonuclease DNA Analysis (BRENDA)	24
	Polymerase Chain Reaction (PCR)	26
	Treatment of Leptospirosis	27
	Control and Prevention of Leptospirosis	28
	Leptospirosis in Cattle in Malaysia	30
	Survival of Leptospire Outside the Host	32
	Leptospire Found in Waters and Soils	33



3	INVESTIGATION OF <i>LEPTOSPIRA INTERROGANS</i> SEROVAR <i>HARDJO</i> FROM CATTLE, WATER AND SOIL SAMPLES IN CATTLE FARMS	
	Introduction	36
	Materials and Methods	41
	Animals	42
	Water and Soil Samples	42
	Serological Examination	42
	Bacterial Culture	44
	Classification of Leptospiral Isolates	45
	Serogrouping of Leptospiral Isolates	47
	Characterization of Leptospire by Bacterial Restriction Endonuclease DNA Analysis	47
	Characterization of Leptospire Based on their Protein Profiles and Immunogenicity	50
	Results	56
	Serology	56
	Bacteriology	57
	Classification of Leptospiral Isolates	59
	Identification of Leptospiral Isolates	60
	Discussions	75
4	EXPERIMENTAL INFECTION OF <i>LEPTOSPIRA INTERROGANS</i> SEROVAR <i>HARDJO</i> OF MALAYSIAN ISOLATE IN INDIGENOUS CALVES	
	Introduction	84
	Materials and Methods	88
	Experimental Animals	88
	Experimental Inoculum	88
	Experimental Procedure	88
	Urine and Blood Sampling	89
	Direct Examination of Blood and Urine Samples by Dark-field Microscopy	90
	Bacterial Culture	90
	Polymerase Chain Reaction (PCR)	92
	Hematological Analysis	95
	Serological Examination	96
	Histopathological Examination	96
	Identification of serovar <i>hardjo</i> Reisolated from Experimental Cattle by Restriction Endonuclease DNA Analysis (BRENDA)	100

Results	103
Clinical Signs	103
Cultural Findings	103
Haematological Findings	104
Histological Findings	105
Bacterial restriction endonuclease analysis (BRENDA)	109
Serological Findings	109
PCR	111
Discussions	114
5 THE SURVIVAL OF <i>LEPTOSPIRA INTERROGANS</i> SEROVAR <i>HARDJO</i> IN THE MALAYSIAN ENVIRONMENT	
Introduction	125
Materials and Methods	129
Bacterial Strains and Inoculum	129
Water, Soil and Bovine Urine Samples	129
Experimental Conditions and Procedures	130
Survival Time of serovar <i>hardjo</i> in Waters	130
Survival Time of serovar <i>hardjo</i> in Soils	130
Survival Time of serovar <i>hardjo</i> in Bovine Urine	131
Leptospiral Culture.....	131
Comparison of Direct Culture and Hamster	
Inoculation	131
Infectivity of serovar <i>hardjo</i> in Water, Soil and Urine	
Samples to Hamsters	132
Results	133
Survival of serovar <i>hardjo</i> in Water	133
Survival of serovar <i>hardjo</i> in Soils	134
Survival of serovar <i>hardjo</i> in Bovine Urine	135
Comparison in Isolation of serovar <i>hardjo</i> from Water,	
Soil and Urine Samples Using Direct Culture and Hamster	
Inoculation	136
Infectivity of serovar <i>hardjo</i> in Water, Soil and Urine	
Samples to Hamsters	138
Discussions	141

6	EXPERIMENTAL TRANSMISSION OF <i>LEPTOSPIRA INTERROGANS</i> SEROVAR <i>HARDJO</i> FROM ENVIRONMENT TO ANIMAL MODEL	
	Introduction	151
	Materials and Methods	155
	Bacterial Strain and Inoculum	155
	Experimental Animals	155
	Transmission of serovar <i>hardjo</i> from Contaminated Water to Experimental Animals	156
	Transmission of serovar <i>hardjo</i> from Contaminated Soil to Experimental Animals	156
	Control Animals	157
	Clinical Observation	157
	Isolation of serovar <i>hardjo</i> from Blood	157
	Serological Examination	158
	Isolation of serovar <i>hardjo</i> from Kidney	158
	Histopathology	159
	Results	159
	Clinical Signs	159
	Isolation of serovar <i>hardjo</i> from Blood and Kidney of Experimental Animals	159
	Serological Findings	161
	Histopathological Finding	163
	Discussions	165
7	GENERAL DISCUSSION AND CONCLUSION	169
	BIBLIOGRAPHY	183
	APPENDICES	204
	VITA	227



LIST OF TABLES

Table		Page
3.1	Isolation of leptospire according to water types.	58
3.2	Isolation of leptospire according to soil types.	58
3.3	The groups of DNA patterns of leptospiral isolates isolated from water and soil in cattle farms digested by <i>EcoR1</i> restriction enzyme.	71
4.1	Isolation of leptospire from blood, urine and kidneys of the experimental animals.	104
4.2	Total white cell count (leukocytes) of experimental cattle inoculated by conjunctiva route and intravenous route.	105
4.3	Serological response (MAT) of the experimental animals to serovar <i>hardjo</i> infection.	110
5.1	The survival time of serovar <i>hardjo</i> in different types of waters under direct sun (32°C) and shaded area (27°C).	134
5.2	The survival time of serovar <i>hardjo</i> in different types of soils under direct sun (32°C) and shaded area (27°C).	135
5.3	The survival time of serovar <i>hardjo</i> in diluted and undiluted urine under direct sun (32°C), shaded area (27°C) and refrigerator (4°C).	136
5.4	Comparison in isolation of serovar <i>hardjo</i> in water samples using direct culture and hamster inoculation.	137
5.5	Comparison in isolation of serovar <i>hardjo</i> in soil samples using direct culture and hamster inoculation.	137
5.6	Comparison in isolation of serovar <i>hardjo</i> in urine samples using direct culture and hamster inoculation	138



6.1	Isolation of serovar <i>hardjo</i> from blood of hamsters exposed to water and soil inoculated with the organism.	160
6.2	Isolation of serovar <i>hardjo</i> from kidney of hamsters exposed to water and soil inoculated with the organism.	160
6.3	Serological finding of serovar <i>hardjo</i> infection in hamsters exposed to soil and water inoculated by the organism.	162



LIST OF FIGURES

Figure		Page
3.1	Protein profiles of leptospiral isolates from waters on cattle farm. Lanes: 1 Protein molecular size markers; 2. Isolate UPM1; 3. Isolate UPM4; 4. Isolate UPM 6; 5. Isolate UPM7; 6. Isolate UPM9; 7. Isolate UPM 10; 8. Isolate UPM13; 9. Isolate W4; 10. Isolate W8; 11. Isolate W9; 12. Isolate W11; 13. serovar <i>hardjo</i> reference strain. Numbers on the left indicate molecular size in kilodalton (kD).	64
3.2	Protein profiles of leptospiral isolates from waters on cattle farm. Lanes: 1 and 14. Protein molecular size markers; 2. Isolate 42; 3. Isolate 43; 4. Isolate 51; 5. Isolate 52; 6. Isolate 112; 7. Isolate 113; 8. Isolate 123; 9. Isolate 134; 10. Isolate 151; 11. Isolate 152; 12. Isolate BR25; 13. serovar <i>hardjo</i> reference strain. Numbers on the left indicate molecular size in kilodalton (kD).	65
3.3	Protein profiles of leptospiral isolates isolated from soil in cattle farm. Lanes: 1. Protein molecular size markers; 2. Isolate UPM19; 3. Isolate 4B; 4. Isolate 5A; 5. Isolate 12E; 6. Isolate 13A; 7. Isolate 13B; 8. Isolate 13C; 9. Isolate 13D; 10. Isolate S5; 11. Isolate S6; 12. Isolate S9; 13. serovar <i>hardjo</i> reference strain. Numbers on the left indicate molecular size in kilodalton (kD).	66



- 3.4 Electrophoretic blots of leptospiral isolates from waters and soils detected by serovar *hardjo* antisera. Lanes: 1 and 36. Protein molecular size markers; 2. Isolate 42; 3. Isolate 43; 4. Isolate 51; 5. Isolate 52; 6. Isolate 112; 7. Isolate 113; 8. Isolate 123; 9. Isolate 134; 10. Isolate 151; 11. Isolate 152; 12. Isolate BR25. 13. Isolate 42; 14. Isolate 43; 15. Isolate 51; 16. Isolate 52; 17. Isolate 112; 18. Isolate 113; 19. Isolate 123; 20. Isolate 134; 21. Isolate 151; 22. Isolate 152; 23. Isolate BR25; 24. serovar *hardjo* reference strain; 25. Isolate UPM19; 26. Isolate 4B; 27. Isolate 5A; 28. Isolate 12E; 29. Isolate 13A; 30. Isolate 13B; 31. Isolate 13C; 32. Isolate 13D; 33. Isolate S5; 34. Isolate S6; 35. Isolate S9. Numbers on the right and left indicate molecular size in kilodalton (kD). 69
- 3.5 Restriction patterns of leptospiral isolates digested with *EcoR1*: lanes 1. serovar *hardjo* reference strain; 2. Isolate 152; 3. Isolate 13C; 4. Isolate 13B; 5. Isolate 113; 6. Isolate 112; 7. Isolate 43; 8. Isolate 42; 9. Isolate UPM9; 10. Isolate UPM7; 11. Isolate UPM6; 12. Isolate UPM4; 13. Isolate UPM1; 14. DNA molecular size markers. Numbers on the right indicate molecular weight in kilobase pairs (Kbp). 72
- 3.6 Restriction patterns of leptospiral isolates digested with *EcoR1*: lanes 1 and 14. DNA molecular size markers; 2. serovar *hardjo* reference strain; 3. Isolate BR25; 4. Isolate 52; 5. Isolate 51; 6. Isolate 151; 7. Isolate 134; 8. Isolate 123; 9. Isolate 13A; 10. Isolate 12E; 11. Isolate 5A; 12. Isolate 4B; 13. Isolate UPM 19. Numbers on the right indicate molecular weight in kilobase pairs (Kbp). 73
- 3.7 Restriction patterns of leptospiral isolates digested with *EcoR1*. Lanes: 1. Isolate S5; 2. serovar *hardjo* reference strain; 3. Isolate S6; 4. Isolate UPM13; 5. Isolate UPM13; 6. Isolate UPM10; 7. Isolate 13D; 8 and 9. Incomplete digested DNA; 10. Isolate W11; 11. Isolate S9; 12. Isolate W9; 13. Isolate W8; 14. Isolate W4; 15. DNA molecular size markers. Numbers on the right indicate molecular weight in kilobase pairs (Kbp). 74



4.1	Kidney tissue from animal No. 7892 inoculated by conjunctival route showing mild interstitial mononuclear infiltration (Haematoxylin and eosin 40X).	106
4.2	Liver tissue from animal No. 7892 inoculated by conjunctival route, (a) showing inflammation cells (b) showing degeneration (Haematoxylin and eosin 40X).	106
4.3	Histological sections of normal (a) Kidney (b) Liver (Haematoxylin and eosin 40X).	107
4.4	Immunoperoxidase stained cattle kidney sections (a) Leptospiral antigen stained in infected kidney and (b) Uninfected kidney (Immunoperoxidase-haematoxylin counterstain 40X).	108
4.5	Silver staining of kidney section. Negative to leptospiral organism. (40X).	108
4.6	Restriction <i>EcoR</i> I enzyme patterns of the serovar <i>hardjo</i> inoculum. Lanes: 1. DNA molecular size marker; 2. serovar <i>hardjo</i> isolated from urine of animal No. 7892 that has been inoculated by the conjunctival route; 3. serovar <i>hardjo</i> inoculum.	109
4.7	Gel electrophoresis of PCR products obtained from urine samples seeded with serovar <i>hardjo</i> local isolates. Lanes: 1. PCR product obtained from DNA extraction by phenol extraction method; 2. PCR product obtained from DNA extraction by boiling method; 3. 100 base pairs (bp) molecular marker. An arrow indicates the 240 base pairs DNA fragment that was amplified.	111
4.8	Detection of leptospiral DNA in cattle urine by PCR. Gel electrophoresis of PCR products obtained from urine samples from experimental cattle inoculated with serovar <i>hardjo</i> field isolate. Lanes: 1 to 4. PCR results obtained from urine sample that were positive to leptospire by culture method; 5. 100 base pairs (bp) molecular markers. An arrow indicates the DNA fragment of 240 base pairs that was amplified.	112



4.9	Detection of leptospiral DNA in cattle urine by PCR. Gel electrophoresis of PCR products obtained from urine samples of experimental cattle that were negative to leptospire by culture method. Lane: 1 to 5 PCR results obtained from urine sample that were negative to leptospire by culture method; 6. positive control (serovar <i>hardjo</i>); 7. 100 base pairs (bp) molecular marker.	113
5.1	Antibody titers to serovar <i>hardjo</i> detected by ELISA in hamsters inoculated with water samples.	139
5.2	Antibody titers to serovar <i>hardjo</i> detected by ELISA in hamsters inoculated with soil samples.	139
5.3	Antibody titers to serovar <i>hardjo</i> detected by ELISA in hamsters inoculated with urine samples.	140
6.1	Kidney section from hamster showed mild degeneration and necrosis of the epithelial cells in convoluted tubules. Haematoxylin and eosin 40X.	163
6.2	Silver impregnation method of Warthin-Starry stained leptospire as black dots situated in tubular lumen (arrow). 40X.	164
6.3	Immunoperoxidase stained kidney section of hamster showed leptospire stained as brown colour (arrow). 40X.	164

LIST OF ABBREVIATIONS

ABTS	2, 2-Azino-bis(3-Ethylbenzthiazoline-6-Sulfonic Acid) Diammonium Salt
bp	base pair
BRENDA	Bacterial restriction endonuclease DNA analysis
BSA	Bovine serum albumin
cm	centimeter
DAB	diaminobenzidene
°C	degree celcius
DNA	Deoxyribonucleic acid
EDTA	ethylene diamine tetra acetic
ELISA	Enzyme-linked immunosorbent assay
EMJH	Ellinghausen, McCullough, Johnson and Harris
5-FU	5-fluorouracil
G	gauge
H ₂ O ₂	Hydrogen peroxide
HCl	Hydrochloric acid
H & E	haematoxylin and eosin
HIS	Hyper immune serum
IgG	Immunoglobulin G
IgM	Immunoglobulin M



JS	Johnson and Seiter
Kbp	Kilobase pair
kDa	Kilodalton
M	Molar
MAT	Microscopic agglutination test
MARDI	Malaysian Agricultural Research and Development Institute
mM	Millimolar
ml	Milliliter
mmol/l	milimol per liter
mg/l	milligram per liter
mol/l	milimol per liter
OD	Optical density
PAGE	Polyacrylamide gel electrophoresis
PBS	Phosphate -buffer saline
PCR	Polymerase chain reaction
pH	puissance hydrogen (Hydrogen-ion concentration)
p.i	post inoculation
RBC	Red blood cell
PCV	Pack cell volume
RDP	Rancangan Daging Penusu
rpm	round per minute
SDS	Sodium dodecyl sulfate

TBS	Tris buffer saline
Tris-HCL	Tris (hydroxymethyl) aminomethane hydrochloride
TEMED	N,N,N',N'-Tetra-methylethylenediamine
UPM	Universiti Putra Malaysia
v/v	volume per volume
w/v	weight per volume
WBC	White blood cell
μ	micron
μg	microgram
μl	microlitre