

**UNIVERSITI SAINS MALAYSIA
GERAN PENYELIDIKAN UNIVERSITI PENYELIDIKAN
LAPORAN AKHIR**

**DETECTION, GENOTYPING & TRANSCRIPTOME
ANALYSIS OF S.TYPHI & S PARATYPHI FORM WELL
WATER & ITS ASSOCIATION WITH GEOGRAPHICAL
CLUSTERING ON ENTERIC FEVER**

PENYELIDIK

DR. AZIAH ISMAIL

PENYELIDIK BERSAMA

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PROFESSOR MOHD ZAKI SALLEH
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DR. WAN MANSOR HAMZAH
DR. FAUZIAH MOHD NOR
PUAN W. ROHAILA W. AB LAH
SALWANI MUHAMAD HARISH**

2015



FINAL REPORT
EXPLORATORY RESEARCH GRANT SCHEME (ERGS)
Laporan Akhir Skim Geran Penyelidikan Eksploratori (ERGS) IPT
Pindaan 1/2015

RESEARCH TITLE: Detection, genotyping and transcriptome analysis of *S. Typhi* and *S. Paratyphi* from well water and its association with geographical clustering of enteric fever

PHASE & YEAR: PHASE 1/2012

START DATE: 1 AUGUST 2012

END DATE: 31 JULY 2014

EXTENSION PERIOD (DATE): RMC LEVEL: 1 AUGUST 2014 – 31 JANUARY 2015
 KPM LEVEL: 1 FEBRUARY – 31 APRIL 2015

PROJECT LEADER: DR AZIAH ISMAIL

I/C / PASSPORT NUMBER: 691206-03-5168



PROJECT MEMBERS:

1. ASSOC. PROF. PHUA KIA KIEN; 2. DR VENUGOPAL A/L BALAKRISHNAN; 3. PROFESSOR MOHD ZAKI SALLEH; 4. DR AHMAD FILZA ISMAIL; 5. DR WAN MANSOR HAMZAH; 6. DR. FAUZIAH MOHD NOR; 7. PUAN W ROHAILA W AB LAH; SALWANI MUHAMAD HARISH
 (including GRA)

PROJECT ACHIEVEMENT (*Prestasi Projek*)

ACHIEVEMENT PERCENTAGE

Project progress according to milestones achieved up to this period	0 - 50%	51 - 75%	76 - 100%
Percentage			√

RESEARCH OUTPUT

Number of articles/ manuscripts/ books (Please attach the First Page of Publication)	Refereed Journal	Non-Refereed Publication
	2 Journal articles (To be submitted) 2 GenBank submission 2 Journal proceedings	
Conference Proceeding (Please attach the First Page of Publication)	International	Regional
	2	1
Intellectual Property (Including Paten, Copyright, Industrial Design, layout Design of Integrated Circuit & Trademarks)	1 (In the process of patent writing)	

HUMAN CAPITAL DEVELOPMENT

Human Capital	Number				Others (please specify)
	On-going		Graduated		
Citizen	Malaysian	Non Malaysian	Malaysian	Non Malaysian	1. Mohd Noramin Ahmad (Technologist) 2. Norhafiza Mohd Nasir (Technologist) 3. Haslizai Hassan (Laboratory Assistant) 4. Norain Haslinie Hasri (Research As
No. PHD STUDENT					
Student Fullname: IC / Passport No: Student ID:					
No. MASTER STUDENT					
Student Fullname: IC / Passport No: Student ID:	MSc (1) Salwani Muhamad Harish IC:900617137466 Std ID: PNFM0005/14R		MPH (1) Dr. Mohd Irwan Maarof IC:810128015135 Std ID: PUM0210/13		
No. UNDERGRADUATE STUDENT					
Student Fullname: IC / Passport No: Student ID:					
Total					

PENDITURE (Perbelanjaan)

Budget Approved (Peruntukan diluluskan) : RM 178,000
Amount Spent (Jumlah Perbelanjaan) : RM 178,000
Balance (Baki) : RM 0.00
Percentage of Amount Spent : 100 %
 (Peratusan Belanja)

ADDITIONAL RESEARCH ACTIVITIES THAT CONTRIBUTE TOWARDS DEVELOPING SOFT AND HARD SKILLS

Aktiviti Penyelidikan Sampingan yang menyumbang kepada pembangunan kemahiran insaniah)

International		
Activity	Date (Month, Year)	Organizer
(e.g : Course/ Seminar/ Symposium/ Conference/ Workshop/ Site Visit)		
National		
Activity	Date (Month, Year)	Organizer
(e.g : Course/ Seminar/ Symposium/ Conference/ Workshop/ Site Visit)	PROGRAM KESEDARAN KESIHATAN PENYAKIT BAWAAN AIR DAN MAKANAN 16 NOVEMBER 2013 KG TELIAR PASIR MAS	INFORMM, USM PPSP, USM JABATAN KESIHATAN KELANTAN PEJABAT KESIHATAN DAERAH PASIR MAS, KELANTAN

E	PROBLEMS / CONSTRAINTS IF ANY (<i>Masalah/ Kekangan sekiranya ada</i>)
	-
F	RECOMMENDATION (<i>Cadangan Penambahbaikan</i>)
	-
G	RESEARCH ABSTRACT – Not More Than 200 Words (<i>Abstrak Penyelidikan – Tidak Melebihi 200 patah perkataan</i>)
	A total number of 936 waters samples were collected from the typhoid locality in the year 2011-2013. <i>S. Typhi</i> was isolated from 2 samples whereby one them are from the outbreak cases in Pasir Mas, Kelantan dan the other one is the sporadic cases. Multiplex PCR showed <i>S. Typhi</i> positives in 19 cases suggesting that the PCR is more sensitive than the culture method. GIS analysis showed model of relationship between water positivity and cases. The <i>S. Typhi</i> isolated from water samples from outbreak area was genotyped together with the <i>S. Typhi</i> isolated from stools of food handlers in the same outbreak area. The pulse-field gel electrophoresis (PFGE) showed similar pattern with the isolates from well water. The findings suggested that the carriers among food handlers had contaminated the water and causing the outbreak. A representative <i>S Typhi</i> isolated from stools of food handlers and one of the <i>S. Typhi</i> isolated from water samples were undergone genome and RNA sequencing. The results showed that there are several genes that can differentiate between the two isolates. Transcriptome analysis showed that 1272 genes were upregulated when <i>S. Typhi</i> was incubated in well water compared to the our genome analysis whereas 1065 genes were upregulated compared to CT18 genome (reference genome sequence in GenBank, NCBI). Further downstream analysis will be continued using other future grants. For overall findings and achievements, please refer to Appendix 1-6 Appendix 1: Research Finding Appendix 2: Research output 1 (Journal proceeding & Conference proceeding) Appendix 3: Research output 2 (Journal articles) Appendix 4: Research output 3 (GeneBank Submission) Appendix 5: Research output 4 (Students) Appendix 6: Research output 5 (Patent)
	 Date : 16 June 2015 Tarikh Project Leader's Signature: Tandatangan Ketua Projek

COMMENTS, IF ANY/ ENDORSEMENT BY RESEARCH MANAGEMENT CENTER (RMC)

(Komen, sekiranya ada/ Pengesahan oleh Pusat Pengurusan Penyelidikan)

Name:

Nama:

Date:

Tarikh:

PROF. DR LEE KEAT TEONG
Pegawai
Pejabat Pengurusan & Kreativiti Penyelidikan
Universiti Sains Malaysia

Signature:

Tandatangan:


19/6/15

Purchase Requisition		Purchase Order		Suppliers		Performance		Francas		Coca In's		Reports		Ar.r.r	
UserCode: NASTR / USMCKCLIVE / CIPPM Current Date : 15/06/2015 4:43:09 PM Version: 15.120, Last Updated at 10/12/2014 Program Code: Votebook9100 DB: 13.00, 09/18/2010 VB: 13.01, 03/14/2011 Switch Language : English / Malay															
Wildcard : eg. Like 100%, Like 10%1, Like %1															
Element 1: 203		Element 2: %		Year: 2015		Element 4: CIPPM									
Element 5: 6730077															
Detail	Excel	Budget Rule	Budget Control	Account Description	Budget Account Code	Roll over	Budget	Cash Received	Advanced	Commit	Actual	Available	Percentage		
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Detail	Excel	302	T	ERGS	203.227.0.CIPPM.6730077	39,448.61	0.00	0.00	0.00	0.00	0.00	39,448.61	0.00%		
Detail	Excel	302	T	ERGS	203.228.0.CIPPM.6730077	970.00	0.00	0.00	0.00	0.00	0.00	970.00	0.00%		
Detail	Excel	302	T	ERGS	203.229.0.CIPPM.6730077	-60,526.75	0.00	0.00	0.00	0.00	0.00	-60,526.75	0.00%		
		302	T	SubTotal		-14,603.14	0.00	0.00	0.00	0.00	0.00	-14,603.14	0.00%		
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		303	T	SubTotal		-12,202.70	0.00	0.00	0.00	0.00	-240.98	-11,961.72	0.00%		
		9999		GrandTotal		-206.24	0.00	0.00	0.00	0.00	-240.98	34.74	0.00%		



Appendix-1: Research findings

Detection, genotyping and transcriptome analysis of *S. Typhi* and *S. Paratyphi* from well water and its association with geographical clustering of enteric fever

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Universiti Sains Malaysia
11 June 2015

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Team members



- Dr Venugopal Balakrishnan, USM
- Assoc. Prof Phua Kia Kien, USM
- Dr Ahmad Filza Ismail, USM
- Prof Mohd Zaki Salleh, UiTM
- Wan Mansor Hamzah, JKNK
- Dr Fauziah Mohd Nor, JKNK
- Dr Hani Mat Husin, JKNK
- Wan Rohaila Wan Ab Lah, JKNK

Introduction



- ❑ Enteric fever is still a serious public health problem in underdeveloped and developing countries.
- ❑ WHO estimates an incidence of 21.6 million typhoid cases with at least 200 000 deaths and 5.4 million paratyphoid cases annually mainly among children (Crump & Mintz, 2010).
- ❑ Enteric fever is caused by *S. Typhi* and *S. Paratyphi* and it is transmitted by eating contaminated food and water handled by food handlers who are carriers.
- ❑ Due to low culture isolation rate from stool (2-5%), carriers will continue to perpetuate the disease.
- ❑ There is an urgent need to increase the chance of carrier detection so as to decrease the risk they pose to the communities.
- ❑ If carriers can be detected and treated, we would be able to effectively control the spread of typhoid.



Fig. 1. Rod shape and flagella are visualized (C) of *S. Typhi* bacteria. Copyright © Dr. Michael Runnauer. All Rights Reserved for personal use.

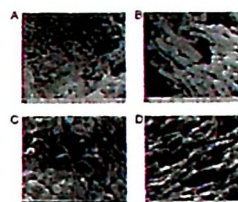
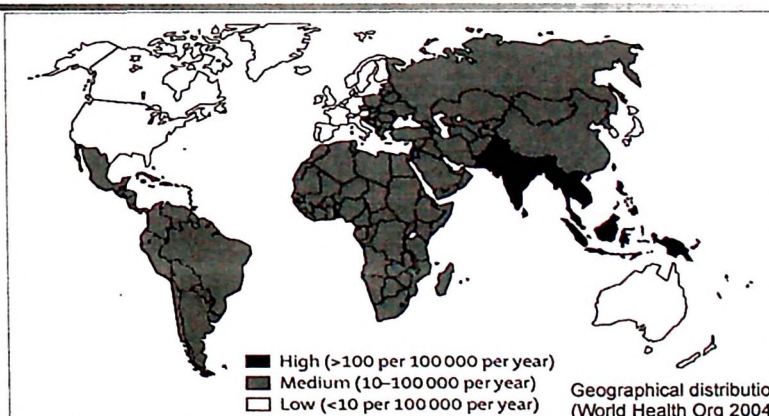


Fig. 2. Cholesterol *S. Typhi* bacteria formation is related to cholesterol production. A) in the laboratory collection of *S. Typhi* images. B) and C) normal bacterial growth on cholesterol. D) growth on cholesterol. E) growth on cholesterol with carbon structures. F) growth on cholesterol with carbon structures. G) growth on cholesterol with carbon structures. H) growth on cholesterol with carbon structures. I) growth on cholesterol with carbon structures. J) growth on cholesterol with carbon structures. K) growth on cholesterol with carbon structures. L) growth on cholesterol with carbon structures. M) growth on cholesterol with carbon structures. N) growth on cholesterol with carbon structures. O) growth on cholesterol with carbon structures. P) growth on cholesterol with carbon structures. Q) growth on cholesterol with carbon structures. R) growth on cholesterol with carbon structures. S) growth on cholesterol with carbon structures. T) growth on cholesterol with carbon structures. U) growth on cholesterol with carbon structures. V) growth on cholesterol with carbon structures. W) growth on cholesterol with carbon structures. X) growth on cholesterol with carbon structures. Y) growth on cholesterol with carbon structures. Z) growth on cholesterol with carbon structures.

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Geographical distribution of typhoid fever



Geographical distribution of typhoid fever (World Health Org 2004; 82: 351)

High incidence:

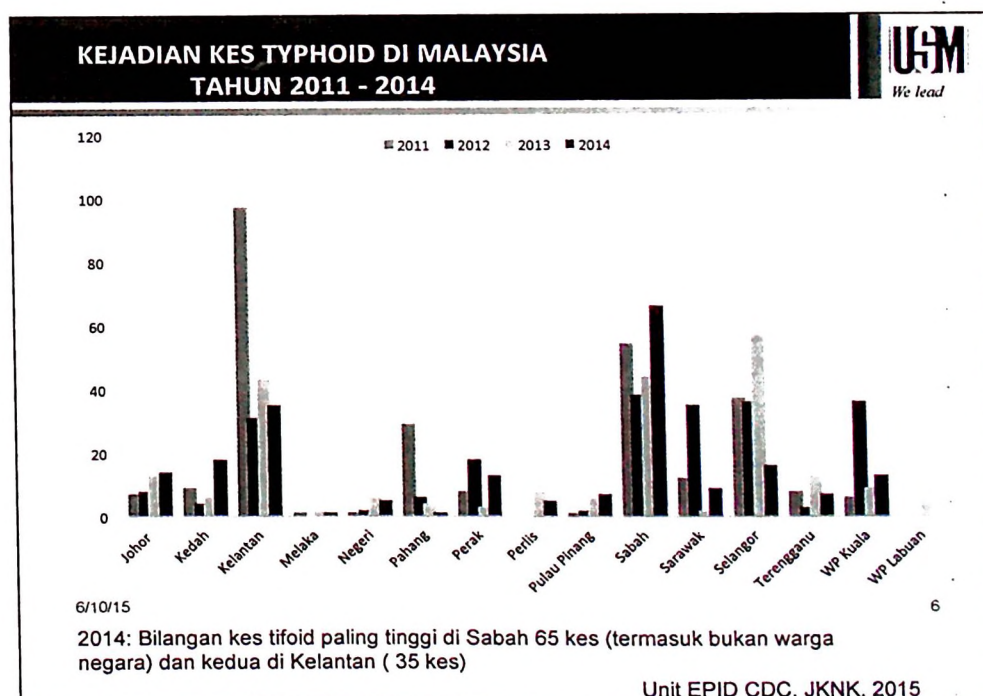
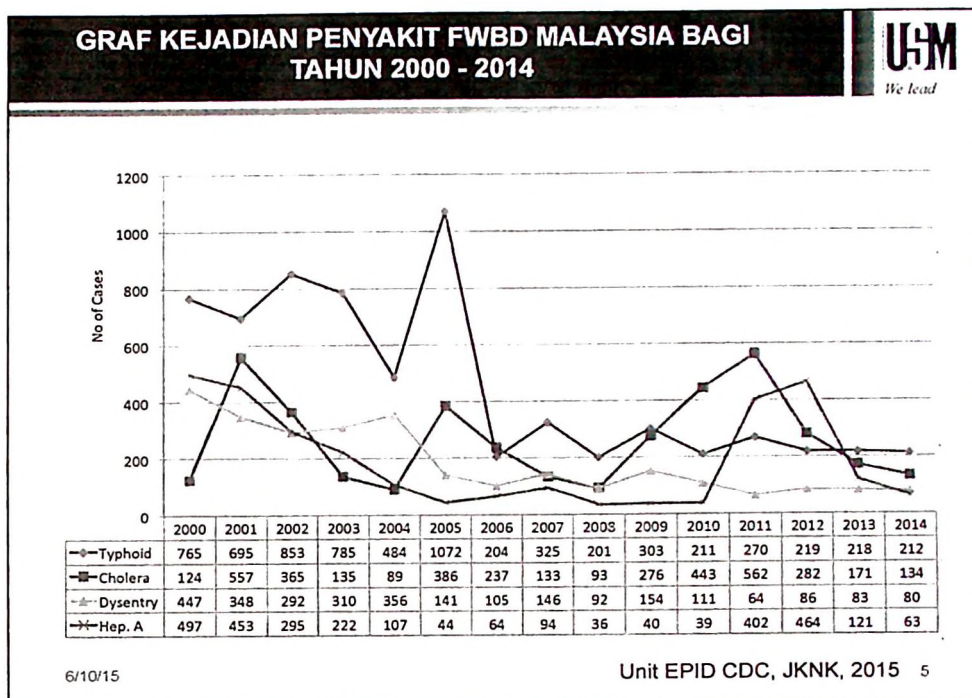
→ South-central Asia (India, Pakistan, Bangladesh)
→ Southeast Asia (Vietnam, Indonesia)

Moderate incidence:

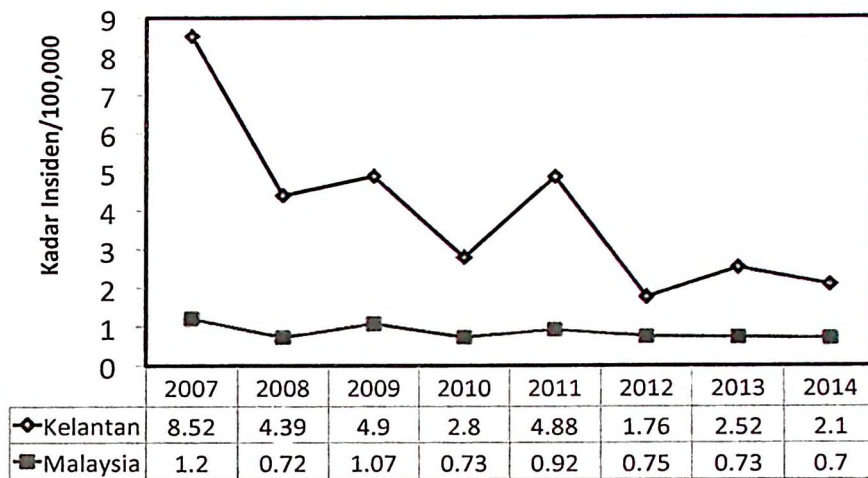
→ The rest of Asia
→ Latin America
→ Africa
→ Oceania

Incidence in Malaysia:

→ < 5/ 100 000 populations



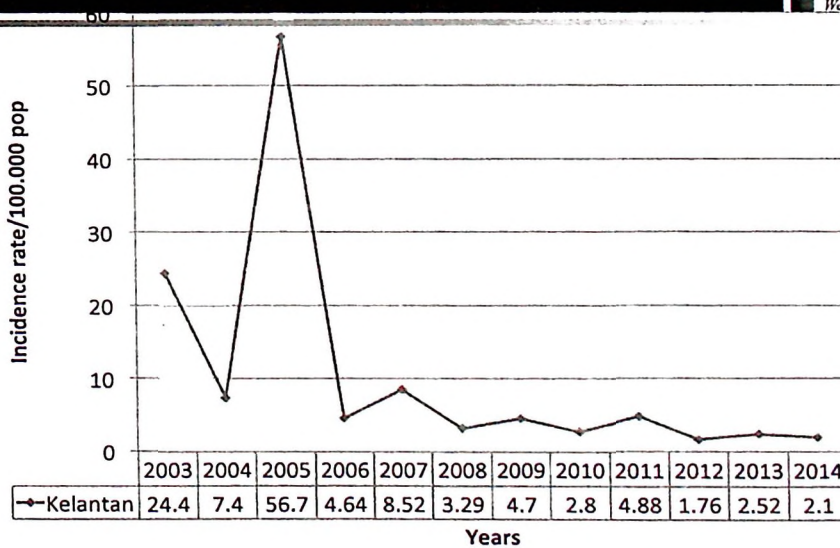
Perbandingan Kadar Insidens Tifoid di Kelantan dan Malaysia; 2007-2014



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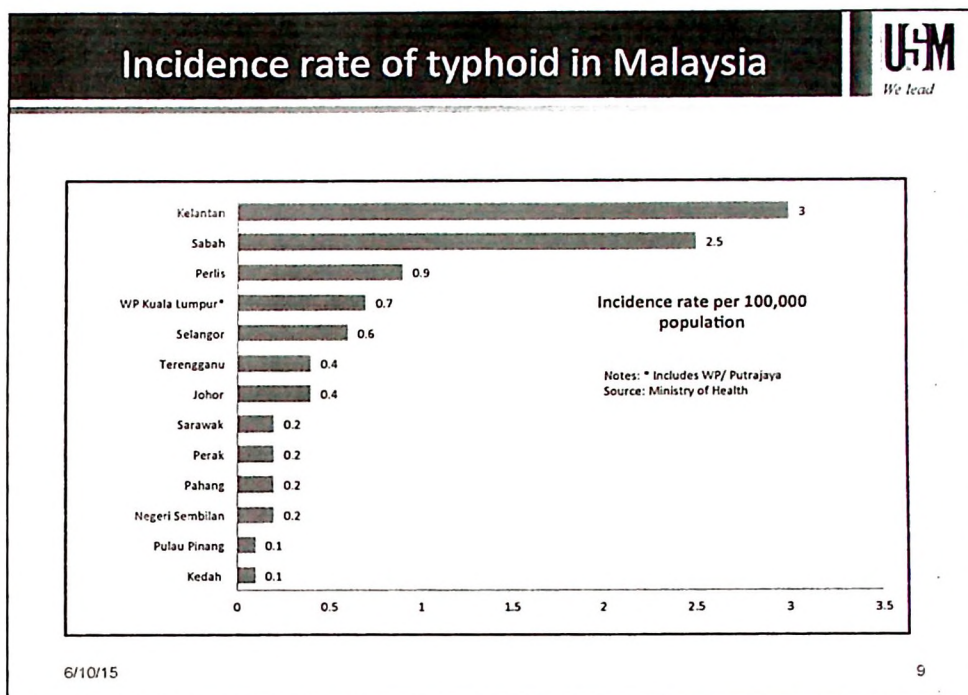
Unit EPID CDC, JKNK, 2015 7

Typhoid Incidence Rate in Kelantan ; 2003 -2014



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Unit EPID CDC, JKNK, 2015 8

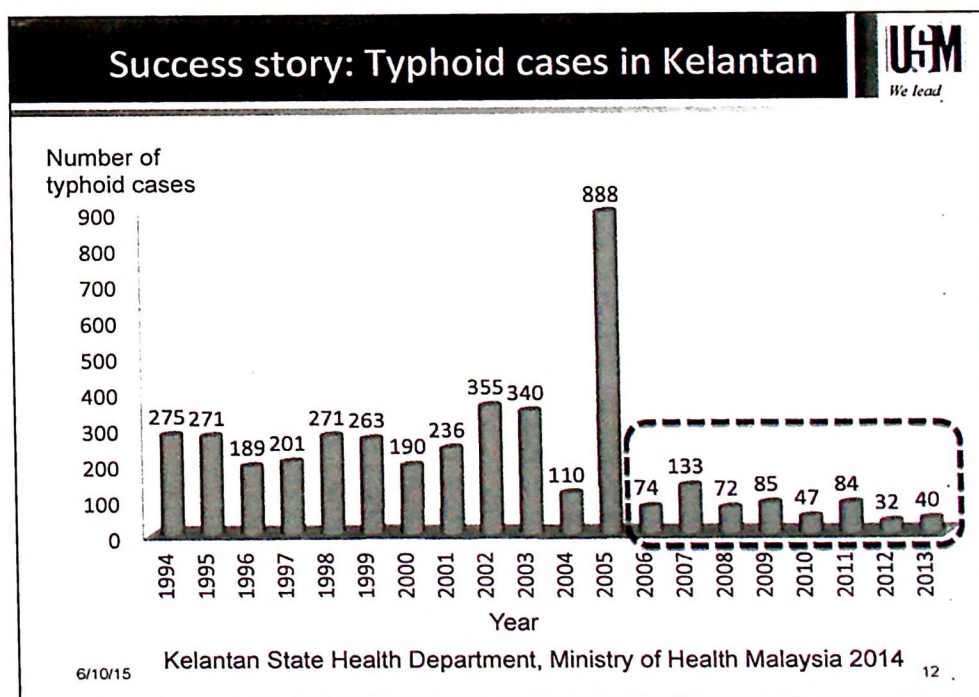
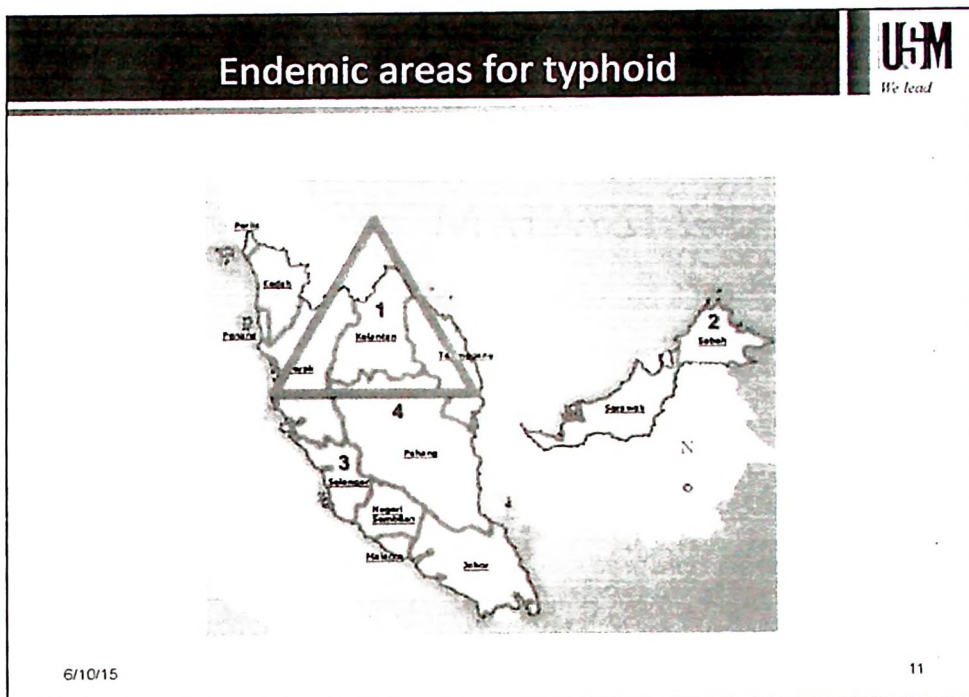


Objectives

U&M
We lead

- General objective:
 - To isolate and investigate Salmonella Typhi and Salmonella Paratyphi from well water associated with enteric fever and carriers
- Specific objectives:
 1. To isolate S. Typhi and S. Paratyphi and detect the bacteria using multiplex PCR dipstick from well water
 2. To genotype using Pulse Field Gel Electrophoresis (PFGE)
 3. To determine the association between bacterial isolates from well water and enteric fever clustering and carriers
 4. To sequence and analyse the bacterial isolates using next generation sequencing platform

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Study locations



We lead

- 5 districts
 1. Pasir Putih
 2. Bachok
 3. Kota Bharu
 4. Pasir Mas
 5. Tumpat



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Detection of bacteria using culture method & molecular method

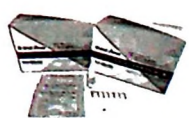
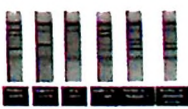


We lead

Water samples from typhoid locality in Kelantan based on the typhoid cases in 2011, 2012 & 2013

N=936 (water samples)	Salmonella Typhi (percentage positivity)	Salmonella Paratyphi A
Culture method	2 (0.2%)	0
Multiplex PCR dipstick	19 (2.0%)	0



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Bacterial genotyping using Pulse Field Gel Electrophoresis (PFGE)



PFGE of chromosomal DNA after digestion with *Xba*I was performed to the total of 8 isolates of *S. enterica* ser. Typhi obtained from the outbreak. PFGE has been widely recommended to differentiate *Salmonella* species (Threlfall *et al.*, 1994)

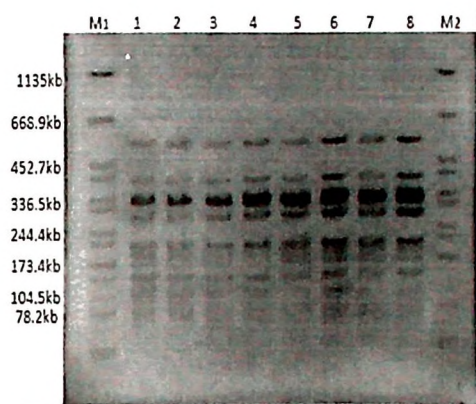


Figure 3: The banding pattern of the eight *S. enterica* ser. Typhi isolates by PFGE. The figure shows the same banding pattern of all eight isolates from food handlers and well water.

Lane M1 and M2: Marker *Salmonella* Braenderup
Lane 1-4, 6-8: Patient and patient's contact sample isolates
Lane 5: Well water sample isolates

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PFGE database

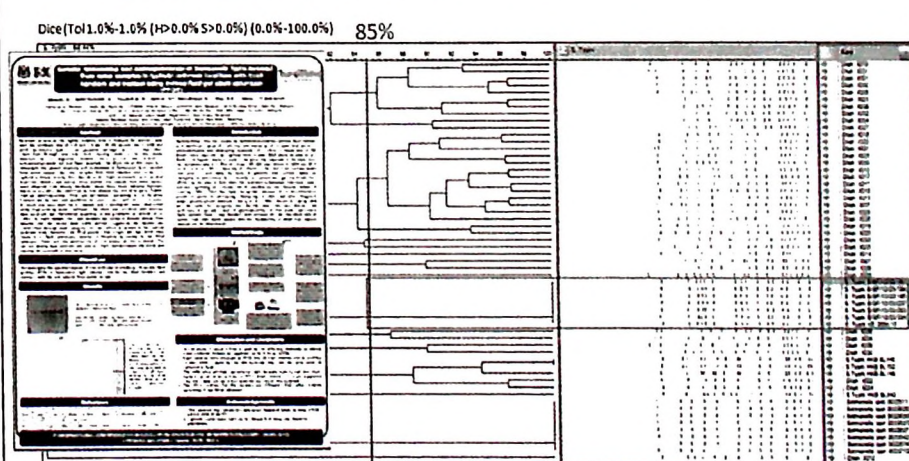
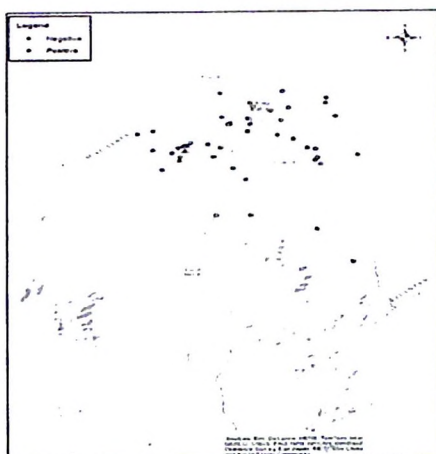


Fig 4: Dendrogram showing the cluster analysis of 49 *S. enterica* ser. Typhi strains identified from database in comparison with 8 new Isolates from this study generated by Fingerprinting™ Quest software by the UPGMA method, based on the matrix of F value which is ≥ 0.85 . The dendrogram also suggested that the isolates obtained from the outbreak did not matched with other pattern from the PFGE database.

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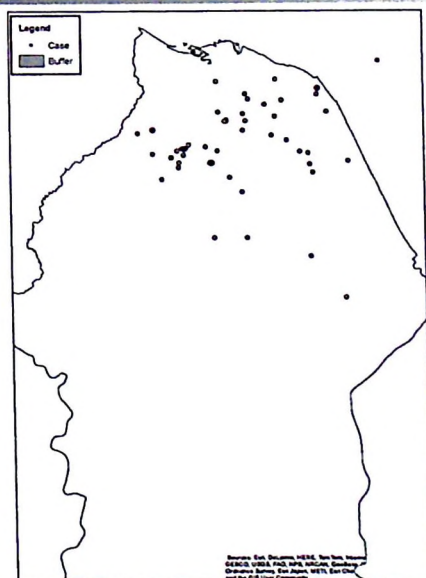
GIS study based on typhoid cases in 2012-2013 : Water samples in typhoid locality



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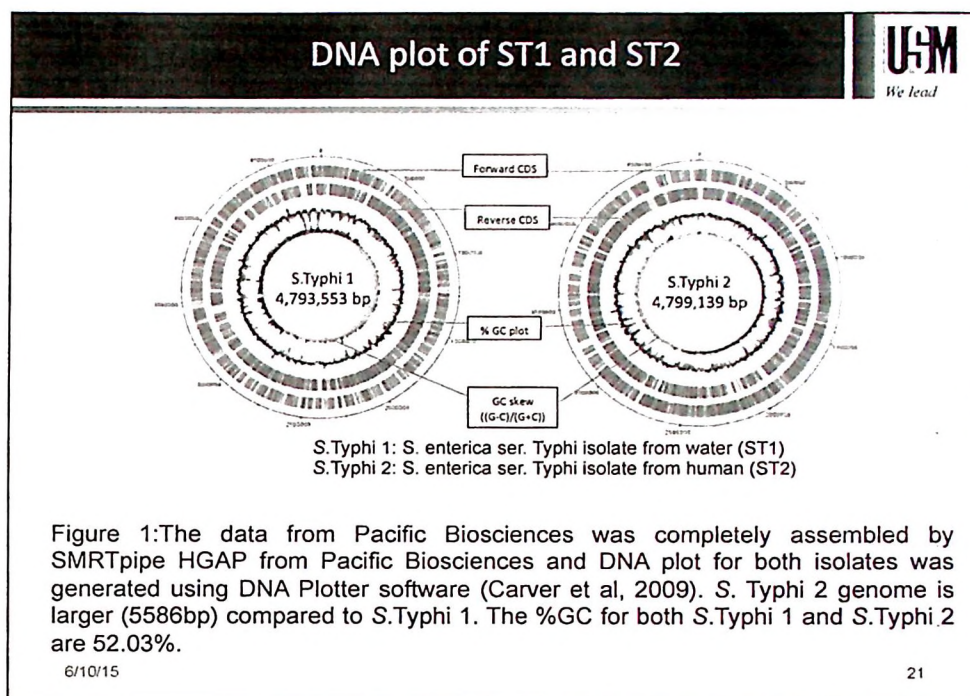
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Intersect water and case buffer 500m



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S.Typhi 1		S.Typhi 2	
ORF	Predicted gene	ORF	Predicted gene
orf00335	substrate-binding transport protein	orf00780	transcriptional regulator c
orf04209	serine threonine-protein phosphatase 6 regulatory subunit 1	orf03103	transcriptional regulator family
orf03006	metalloprotease yggg	orf02056	toxin-module
orf03690	leucine abc transporter substrate-binding partial	orf04116	streptomycin 3'-adenylyltransferase
orf00123	hypothetical protein SEEP9120_11004, partial salmonella	orf04454	protein dsrb
orf06684	hypothetical protein ACS6-2309 E. coli	orf05978	plectovirus svp13 orf 10 transmembrane protein
orf06683	firmicute plasmid replication family protein	orf05407	MULTISPECIES: hypothetical protein [Salmonella]
orf00002	dna-cytosine partial	orf02940	like family protein
orf06534	colanic acid exporter	orf00290	hypothetical protein [Klebsiella pneumoniae]
orf03964	capsular polysaccharide biosynthesis protein	orf02565	galactarate dehydratase
orf06282	capsular synthesis regulator component b	orf00919	flavin reductase
orf04156	atp synthase epsilon chain	orf00950	RF1 atp synthase subunit epsilon
orf00347	aminoglycoside resistance protein	orf01934	dna-invertase lambdoid prophage partial
orf03491	50s ribosomal protein l36	orf01974	cytotoxic and regulatory t-cell molecule
orf00029	50s ribosomal protein	orf03215	cytochrome partial
Table 1: The different of predicted gene in S.Typhi 1 and S.Typhi 2.		orf04453	colanic acid capsular biosynthesis activation protein
		orf05557	chaperone lipoprotein
		orf00579	cellulose synthase regulator protein
		orf02667	cell wall partial
		orf02577	cation diffusion facilitator family transporter
		orf05005	C4-dicarboxylate anaerobic carrier protein
		orf04127	abc substrate-binding family 5
		orf03466	n terminal domain protein
		orf03122	heavy metal sensor kinase subfamily protein

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Assembly result (ST1)

Pre-Assemble

Data	Filtered bp	Read Quality	Seed Bases	PreAssembled bp	PreAssembled Reads	Mean Read Length	Max Read Length
PacBio CLR (1000 4Mbp)	822 4Mbp	0.0	332Mbp	160 621,273	32,975	5,153	15,516

Assembly Results

Data	HGAP Protocol	# of Contigs	Max Contig Length	N50 Contig Length	Total Contig Length	GC Contents (%)
pre-assembled PacBio CLR	HGAP.2	8	4,793,710	4,793,710	4,264,520	51.99

Polishing Results

Data	Polishing Protocol	# of Contigs	Max Contig Length	N50 Contig Length	Total Contig Length	GC Contents (%)
Consensus	Quiver	8	4,793,837	4,793,837	4,264,778	51.99
	Circular	1	4,793,553	4,793,553	4,793,553	52.03

Gene Prediction Results

Scaffold ID	# of ORF	Total ORF Length	Max ORF Length	Min ORF Length
scaffold	4,937	4,176,837	10,875	114

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Assembly result (ST2)

Pre-Assemble

Data	Filtered bp	Read Quality	Seed Bases	PreAssembled bp	PreAssembled Reads	Mean Read Length	Max Read Length
PacBio CLR (771 4Mbp)	584 4Mbp	0.8	150Mbp	116,925,223	21,343	5,478	15,802

Assembly Results

Data	HGAP Protocol	# of Contigs	Max Contig Length	N50 Contig Length	Total Contig Length	GC Contents (%)
pre-assembled PacBio CLR	HGAP.2	2	4,807,468	4,807,468	4,822,784	52.04

Polishing Results

Data	Polishing Protocol	# of Contigs	Max Contig Length	N50 Contig Length	Total Contig Length	GC Contents (%)
Consensus	Quiver	2	4,807,653	4,807,653	4,822,972	52.05
	Circular	1	4,799,139	4,799,139	4,799,139	52.03

Gene Prediction Results

Scaffold ID	# of ORF	Total ORF Length	Max ORF Length	Min ORF Length
scaffold	4,945	4,183,815	10,875	114

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Assembly result (ST2)



Pre-Assembly

Data	Filtered bp	Read Quality	Seed Bases	Pre-Assembled bp	Pre-Assembled Reads	Mean Read Length	Max Read Length
PacBio CLR (772.4Mbp)	544.4Mbp	0.8	180Mbp	116,925,323	21,343	5,478	15,802

Assembly Results

Data	NGAP Protocol	# of Contigs	Max Contig Length	NGO Contig Length	Total Contig Length	GC Contents (%)
pre-assembled PacBio CLR	NGAP.2	2	4,807,468	4,807,468	4,822,724	52.04

Polishing Results

Data	Polishing Protocol	# of Contigs	Max Contig Length	NGO Contig Length	Total Contig Length	GC Contents (%)
Consensus	Quiver	2	4,807,468	4,807,468	4,822,722	52.05
	Circular	1	4,799,139	4,799,139	4,799,139	52.03

Gene Prediction Results

Scaffold ID	# of ORF	Total ORF Length	Max ORF Length	Min ORF Length
scaffold	4,945	4,153,815	10,875	114

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Transcriptome analysis



S.Typhi-1

Comparison	Up Regulation	Down Regulation	Total	Result Files (2. DEG Folder)
1) 2 fold				
1 RNA-B vs RNA-A	1379	1135	2514	1 RNA-B vs RNA-A.1.xlsx (The second sheet)
2) 2fold & pvalue < 0.05				
1 RNA-B vs RNA-A	1272	1082	2354	1 RNA-B vs RNA-A.1.xlsx (The third sheet)
3) 2fold & pvalue < 0.05 & FDR < 0.1				
1 RNA-B vs RNA-A	1272	1082	2354	1 RNA-B vs RNA-A.1.xlsx (The fourth sheet)

S.Typhi-CT18

Comparison	Up Regulation	Down Regulation	Total	Result Files (2. DEG Folder)
1) 2 fold				
1 RNA-B vs RNA-A	1102	1079	2181	1 RNA-B vs RNA-ACT18.xlsx (The second sheet)
2) 2fold & pvalue < 0.05				
1 RNA-B vs RNA-A	1065	1068	2133	1 RNA-B vs RNA-ACT18.xlsx (The third sheet)
3) 2fold & pvalue < 0.05 & FDR < 0.1				
1 RNA-B vs RNA-A	1065	1068	2133	1 RNA-B vs RNA-ACT18.xlsx (The fourth sheet)

Filtering Process

- 1) Fold change > 2-fold
- 2) p-value < 0.05

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Research Output-1: Journal proceedings

(Please refer to Appendix 2)



- **Journal proceedings: 3**

- Salwani, H., Mohd Nuramin, A., Fauziah, M. N., Hani, M. H., Wan, M. H., Phua, K. K., & Aziah, I. (2014). Genetic relationship and correspondence of Salmonella Typhi isolated from water samples in typhoid outbreak localities with food handlers and contact by using Pulsed Field Gel Electrophoresis (PFGE). *Asian Pacific Journal of Tropical Disease*, 4(3), 242. (Journal Proceeding) (Non-ISI)
- Nor, Amalina Z., H. Salwani, H. Norain Haslinie, A. Mohd Nuramin, N. Norhafiza, H. Haslizai, Asma, I. and Aziah, I. "The evaluation of a multiplex PCR-DNA dipstick assay with culture method and EZ Typhi Carrier DNA assay to detect Salmonella Typhi in well water samples." *Asian Pacific Journal of Tropical Disease* 4, no. 3 (2014): 243. (Journal Proceeding) (Non-ISI)
- Mohd Irwan Maarof, Ahmad Filza Ismail, NorSa'adah Bachok, Aziah Ismail, Fauziah Mohd Noor. SPATIAL DISTRIBUTION OF ENTERIC FEVER AND WATER SOURCE POSITIVITY. 46th Asia-Pacific Academic Consortium for Public Health (APACPH) Kuala Lumpur. 17-19 October, 2014. (Proceeding)

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Research Output-2: Journal articles

(Please refer to Appendix 3)



- **Journal proceedings: 2**

- Article 1: Salwani Harish, Kee Shin Sim, Mohd Nazalan Mohd Najimuddin & Ismail Aziah. Genome sequence of Salmonella enterica serovar Typhi isolate PM016/13 from well water associated with a typhoid outbreak in Pasir Mas, Kelantan, Malaysia. (To be submitted to Genome Announcement [American Society for Microbiology (ASM) Journal])
- Article 2: Salwani Harish, Kee Shin Sim, Fauziah Mohd Nor, Hani Mat Hussin, Wan Mansor Hamzah, Mohd Nazalan Mohd Najimuddin & Ismail Aziah. Complete genome sequence of Salmonella enterica subsp. Enterica serovar Typhi isolate B/SF/13/03/195 Associated with a Typhoid Carrier in Pasir Mas, Kelantan, Malaysia. (To be submitted to Genome Announcement [American Society for Microbiology (ASM) Journal])

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Research Output-3: GenBank submission

(Please refer to Appendix 4)



- **Genbank submission: 2 (in the process of submission)**
 - Genome sequencing data of *Salmonella enterica* subsp. Enterica serovar Typhi (B/SF/13/03/195)
 - Submission No: SUB974616
 - Bio Project Accession: PRJNA286162
 - Genome sequencing data of *Salmonella enterica* subsp. Enterica serovar Typhi (PM016/13)
 - Submission No: SUB972538
 - Bio Project Accession: PRJNA286155

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Research Output-4: Students

(Please refer to Appendix 5)



- **Students: 2 (1 MSc; 1 MPH)**
 1. Dr Mohd Irwan Maarof (MPH) – graduated in 2014
 2. Salwani Mohamed Harish (MSc) – in progress

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Research Output-5: Patent

(Please refer to Appendix 6)



- In the process of finalizing patent document
 - Patent application was approved by USM committee

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Project milestone



No	Project activities	Achievement
1	Completion of sample collection	100%
2	Completion of Salmonella isolation	100%
3	Completion of bacteria detection by multiplex PCR	100%
4	Completion of bacterial typing by PFGE	100%
5	Completion of GIS study	100%
6	Completion of genome and transcriptome sequencing	100%
7	Completion of sequencing analysis	100%
8	Completion of report/journal articles writing	100%

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