Method optimisation for detection of *Salmonella* Typhi from the environment

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Malawi background & rationale

Malawi is an typhoid endemic country

- Outbreak of unknown origin in Blantyre, Malawi started 2012
- >16,000 cases per year, ~200 deaths associated with typhoid

Methods Development Project

Primary aim: culture & detect S. Typhi from the environment

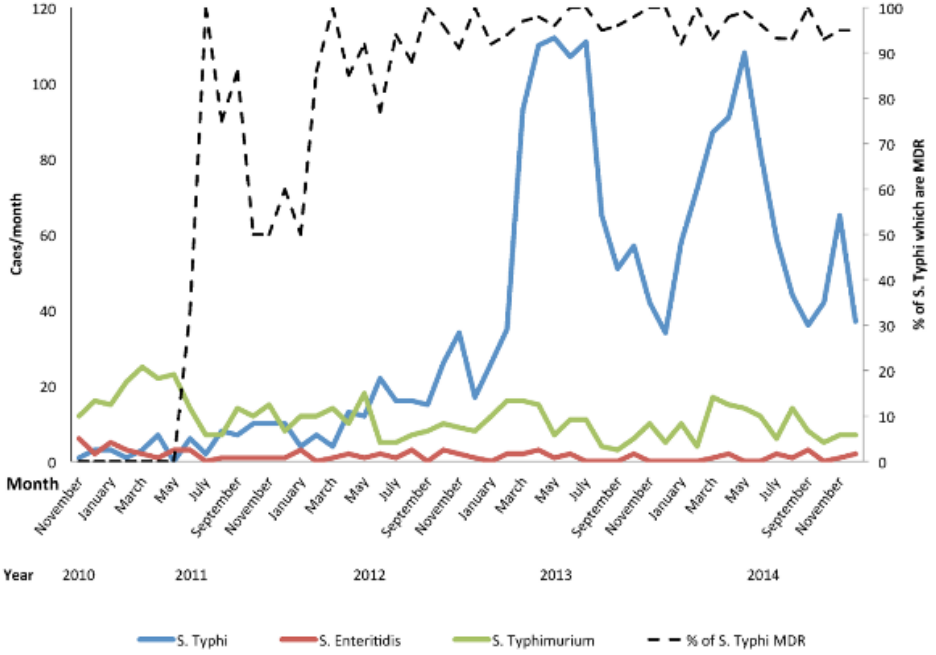


Figure 1: Feasey *et al.*, 2015, Monthly trends in bloodstream invasive Salmonella diagnosed at QECH from November 2010-October 2014.

Isolation of *Salmonella* Typhi from the environment is difficult but not impossible

Sampling from sewerage proved effective

- ≤1950s
  - Moore's swabs
  - Specific required culture methods
- 1980s
  - Reinforced the use of Moore's swabs
  - Selenite-F is the most effective for S. Typhi
- Viable but non-culturable (VBNC)

New approaches use molecular methods

- Strongly associated with water & indirect transmission in endemic regions

Culture remains important

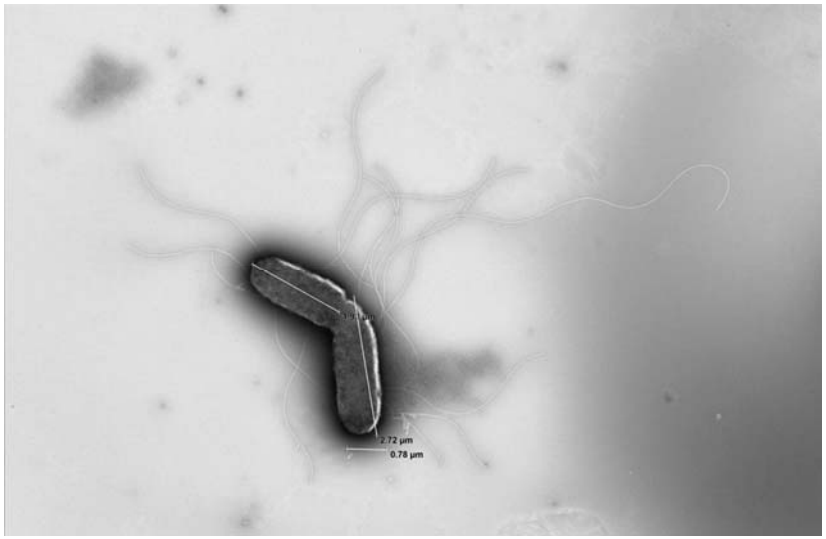


Figure 1: Electron micrograph of a salmonella taken by Matthew Hannah, PHE

Twenty isolates from the PHE culture collection were used

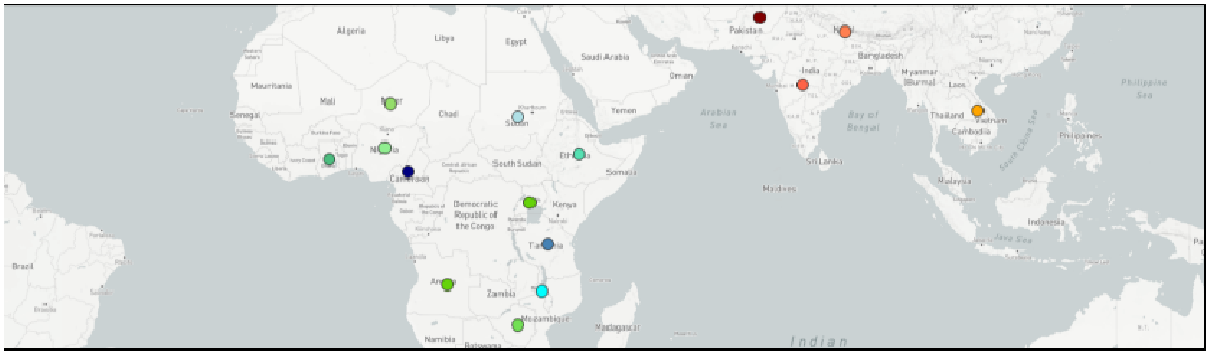


Figure 2: Global distribution of strains used in optimisation & evaluation of environmental isolation (Satheesh Nair, PHE)

Isolates from different regions selected for laboratory testing

The use of a novel chromogenic agar, mCASE was selected for the isolation of salmonella

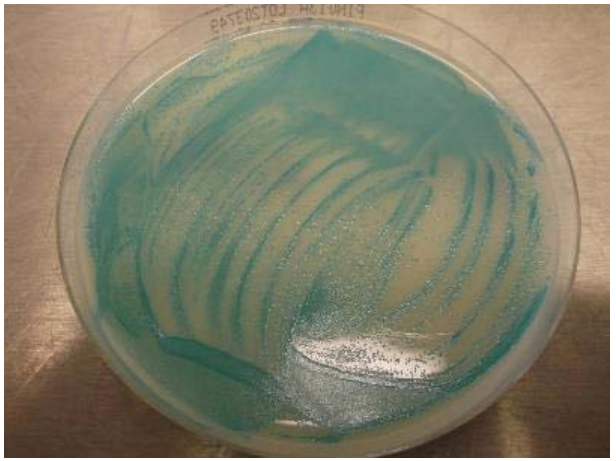


Figure 3: Pure *S.* Typhi on mCASE

Methods for the isolation of *Salmonella* Typhi were assessed

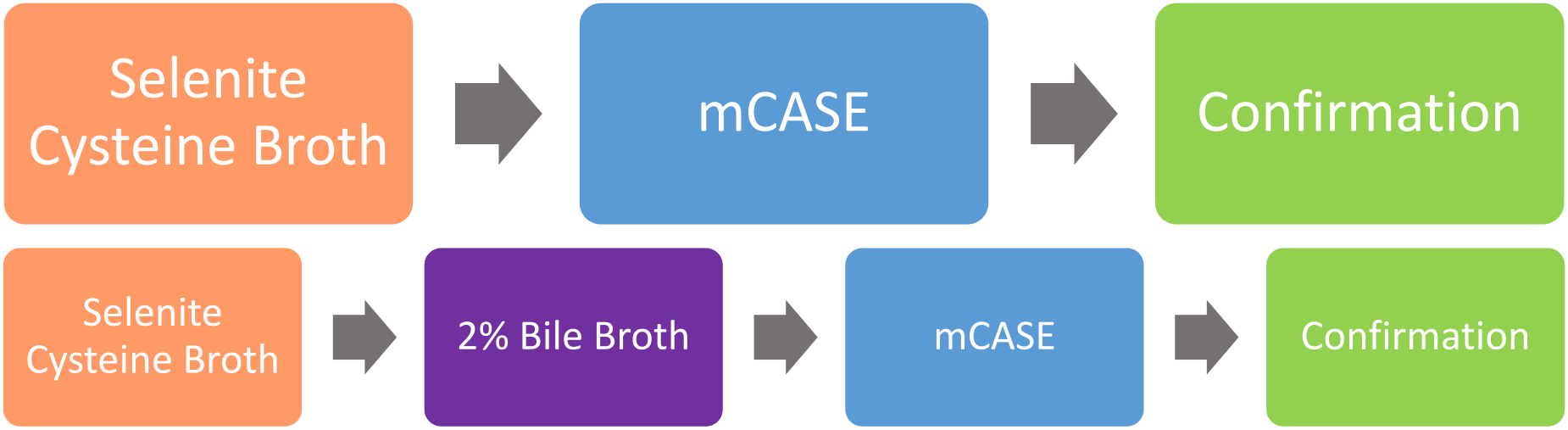


Figure 4: Two examples of the pathways assessed

- Pathways were narrowed to four options from original 15
  - Selenite cysteine broth was preferred due to its selectivity but does not remove *S*. Typhi like alternatives (e.g. Rappaport Vassiliadis broth)
  - Bile broth: infection starts post exposure to bile

Too Many Plates

Preliminary working at PHE Food, Water & Environmental Laboratory

- Hundreds of plates a week processed
  - ❖ Under laboratory conditions with control strains, *S*. Typhi was reliably retrieved
  - ❖ Environmental testing required

Photo Credit: https://www.flickr.com/photos/erikaleef

Following narrowing of the pathways, challenges set up with blind, mixed cultures

Challenge Organisms

- *Salmonella* Nottingham – same colour as *S*. Typhi on mCASE
- *Bacillus cereus* – similar colour & overgrows
- Fungi - blind cultures grew relatively well in the broths

Immuno-magnetic bead separation ran in parallel

- Pan-*Salmonella* bead, developed by Ezzeddine Elmerhebi, Neogen LabM
- Further work required *in situ* as artificial mixtures showed no major difference in recovery rates

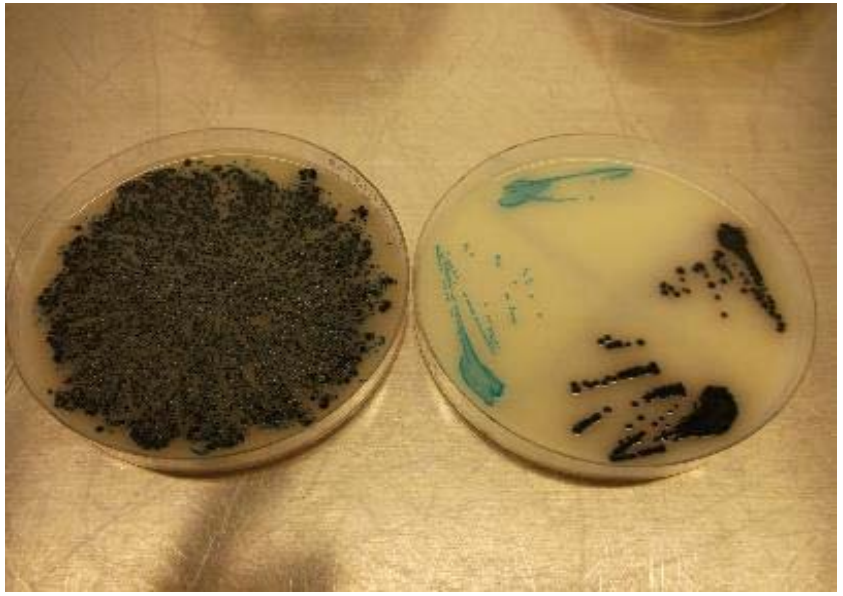


Figure 5: Mixed culture on mCASE, where *S*. Typhi was successfully selected

Molecular approaches utilised to reinforce culture methods developed

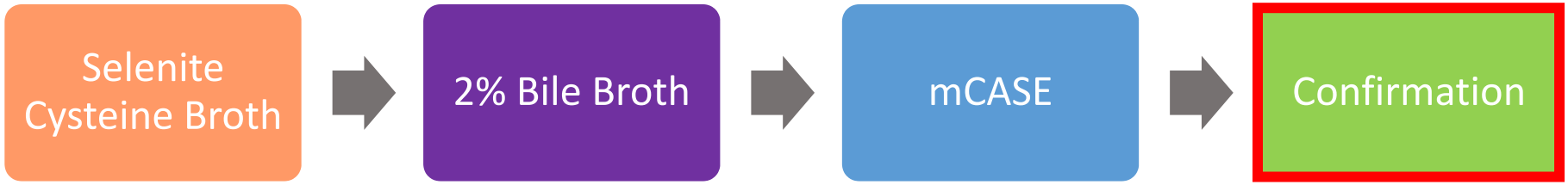


Figure 6: Pathway emphasising confirmation step

- The main aim of the project is to develop a method for culturing environmental *S*. Typhi
- Molecular methods are more cost effective & high-throughput
- Quantitative PCR also allows for screening of incoming samples
  - Inhibitors such as environmental, chemical & faecal contaminants

Note: Current DNA-based molecular methods cannot prove viability

Novel assay from PHE used for rapid diagnostics

Satheesh Nair, PHE, designed an assay for diagnostics

- The original assay included primer's for Paratyphi A, B & C
- This project aims to multiplex the *S*. Typhi targets
- Multiplexed assay is currently being optimised
- Probes changed from original to minimise interference in multiplex

Table 1: Primer sequences of PHE assay and source publications

Gene	Name	Sequence 5'-3'	NCBI Accession Number	Reference
ttr	ttr_F	CTCACCAGGAGATTACAACATGG	AF282268	Hopkins, 2009
	ttr_R	AGCTCAGACCAAAAGTGACCATC		
	ttr_P	FAM-CACCGACGGCGAGACCGACTTT-BHQ1		
sseJ	sseJ_F	CGAGACTGCCGATGCATTTA	AF294582	Nair, 2019
	sseJ_R	GTACATAGCCGTGGTGAGTATAAG		
	sseJ_P	YY-TGGAGGCGGCCAGTAATATTGGTT-BHQ1		
tviB	tviB_F	TGTGGTAAAGGAACTCGGTAAA	NC_003198	Nair, 2004
	tviB_R	GACTTCCGATACCGGGATAATG		
	tviB_P	CY3-TGGATGCCGAAGAGGTAAGACGAGA-BHQ2		
staG	staG_F	CGCGAAGTCAGAGTCGACATAG	AL513382	Nga, 2010
	staG_R	AAGACCTCAACGCCGATCAC		
	staG_P	CY5-CATTTGTTCTGGAGCAGGCTGACGG-BHQ2		

High-Resolution Melt (HRM) PCR

The multiplex assay is being adapted to HRM

- Thomas Edwards, LSTM, consulted in assay conversion
  - ❖ Results consistent with *S*. Typhi & non-typhoidal salmonella control strains
  - ❖ Further optimisation & design changes

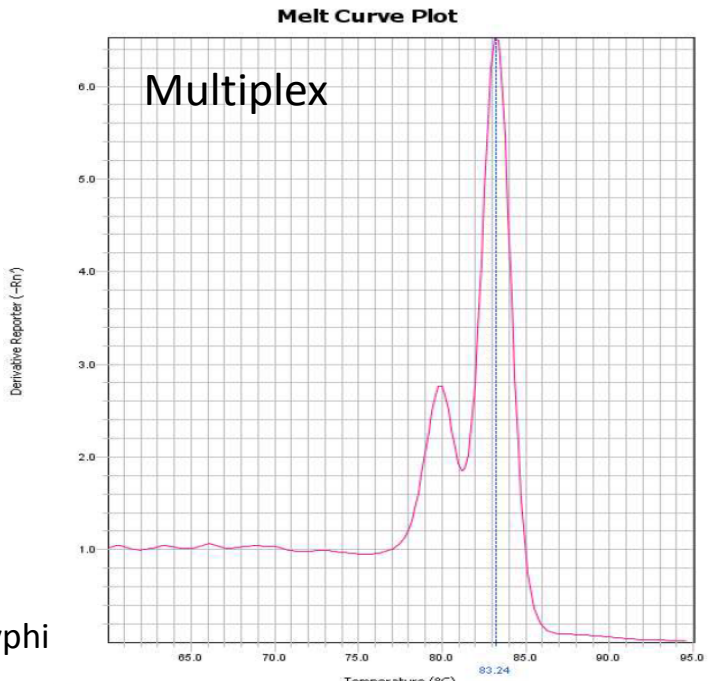
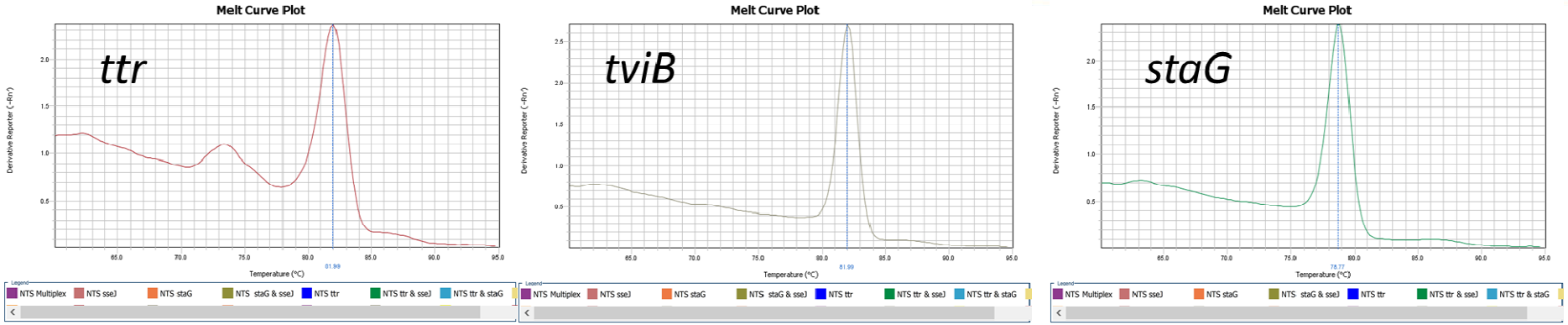


Figure 7: HRM melt curves for positive *Salmonella* Typhi



Role of the assay

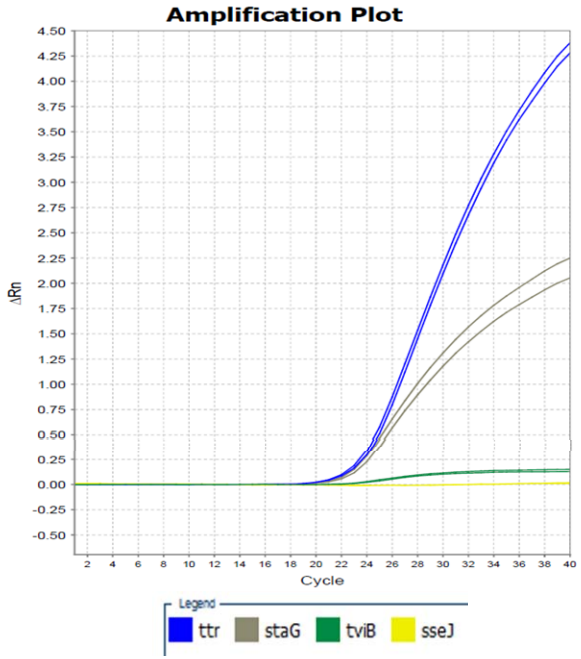


Figure 8: q-PCR assay for blind broth "A"

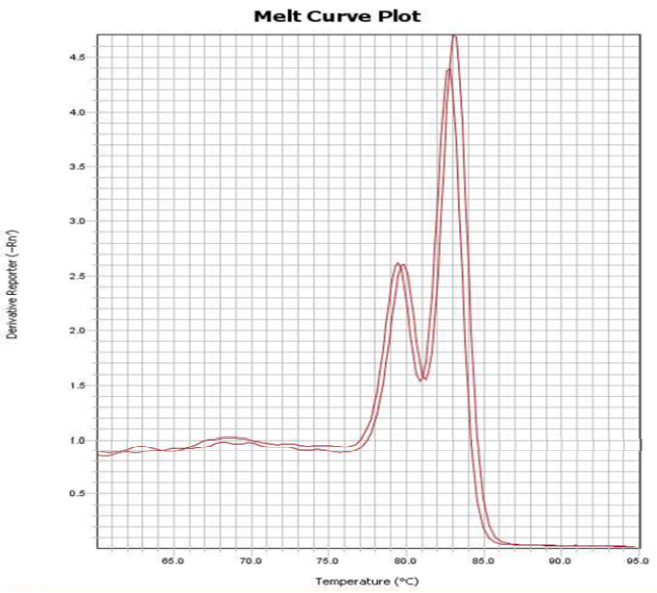


Figure 9: HRM-PCR assay for blind broth "A"

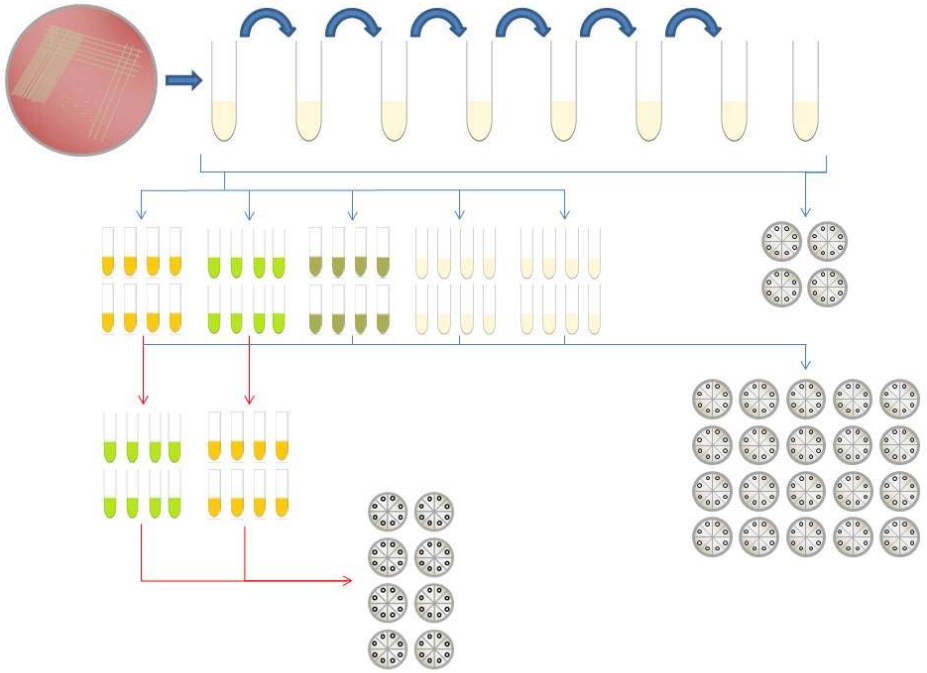
As a tool for screening & confirmation, the blind cultures were processed with both the probe based q-PCR & the HRM PCR

HRM: <£0.40 (<$0.5)
Probe: <£0.50 (<$0.6)

Moving forwards, work to be done in Malawi

Final Culture Pathways

- Limit of detection (LOD) & limit of quantification (LOQ) work still to be performed
- To finalise culture pathways based on further evaluation in Malawi



Moving forwards, work to be done in Malawi

Sampling strategy – in collaboration with Jillian Gauld, University of Lancaster

- Pilot Study
- Challenges
  - ❖ Road conditions & water access
  - ❖ Logistics

Methodology

- Continued optimisation
- Immuno-magnetic separation & microfluidics
- Adapt to new challenges from environmental samples

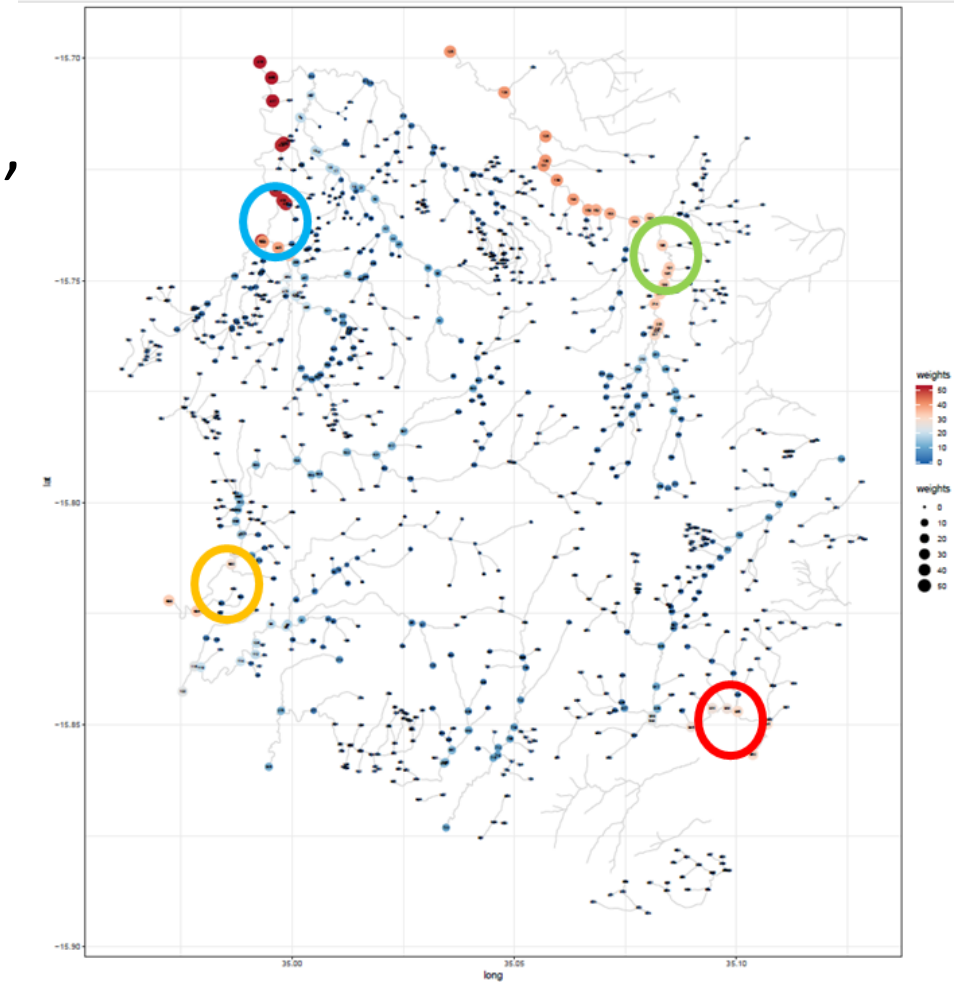


Figure 10: Cumulative map of Typhoid cases with highlighted sampling environmental areas

Likhubula River

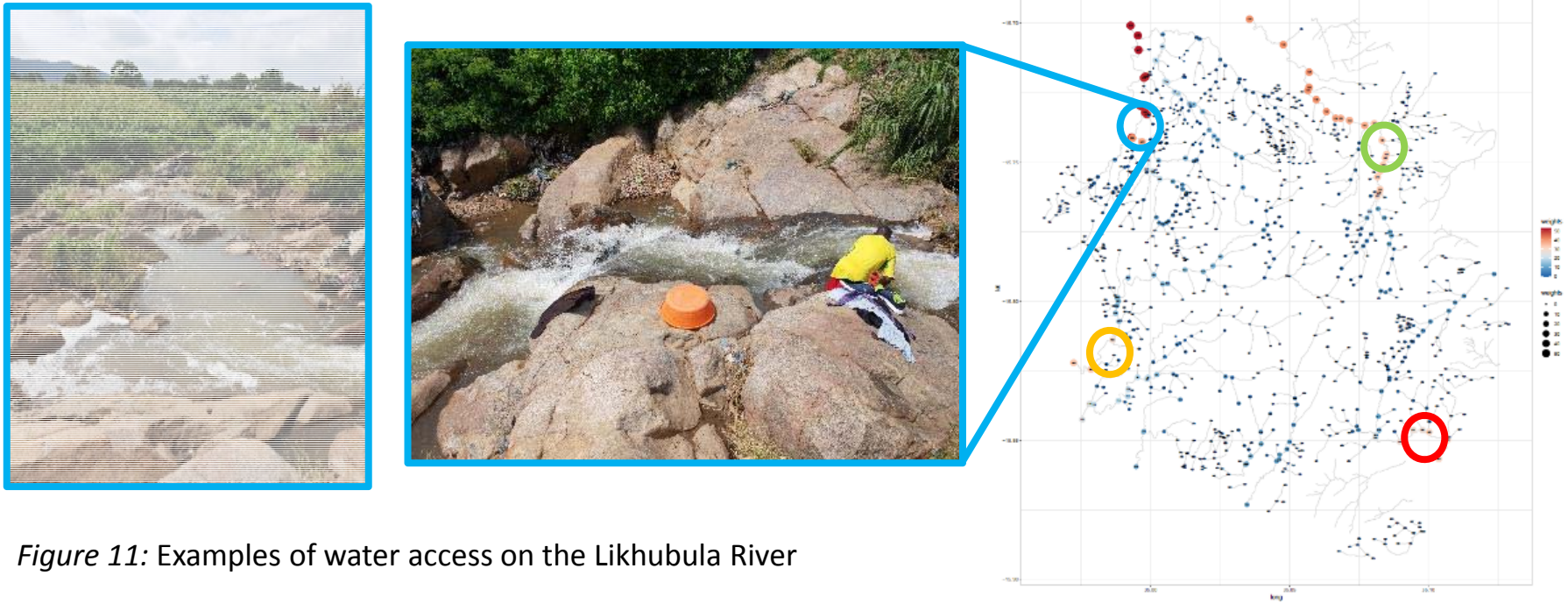


Figure 11: Examples of water access on the Likhubula River

Mudi River

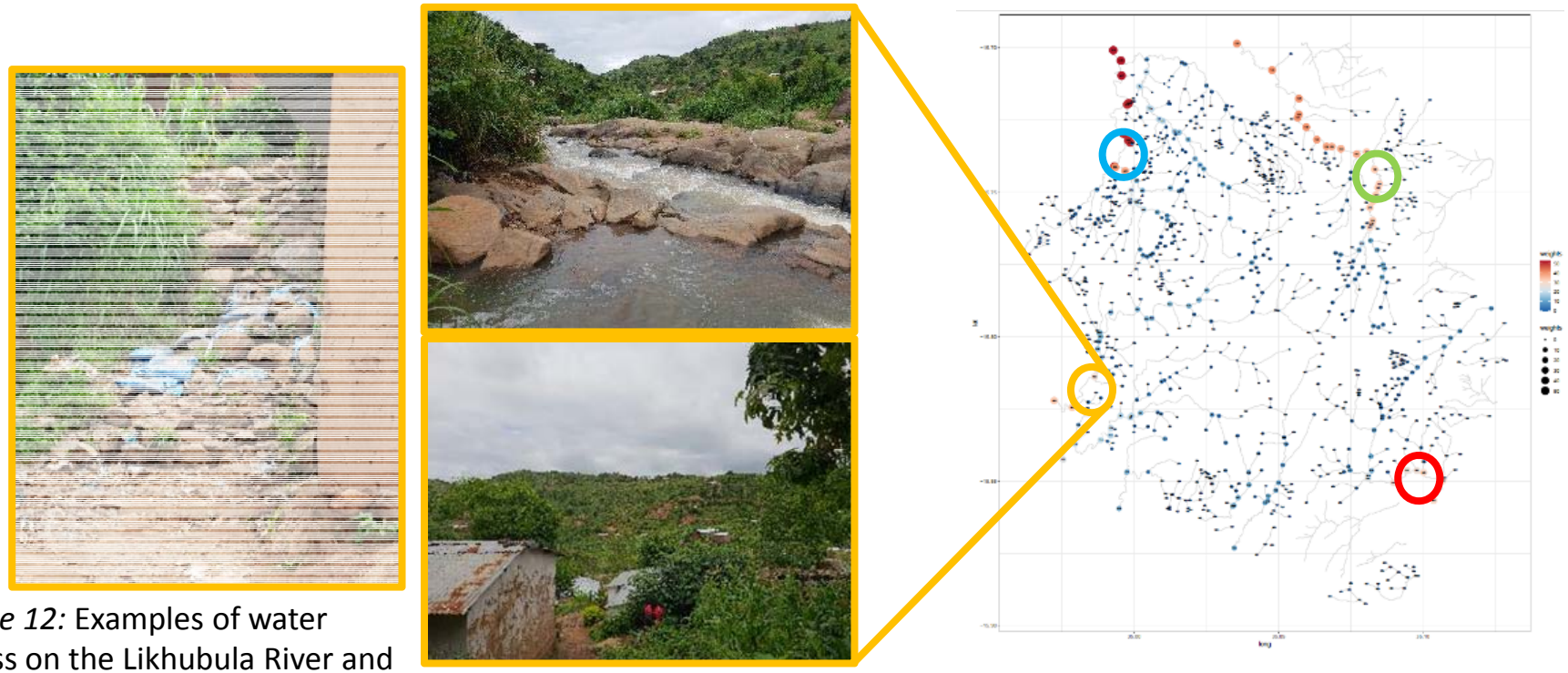


Figure 12: Examples of water access on the Likhubula River and walkway through village

Lunzu River

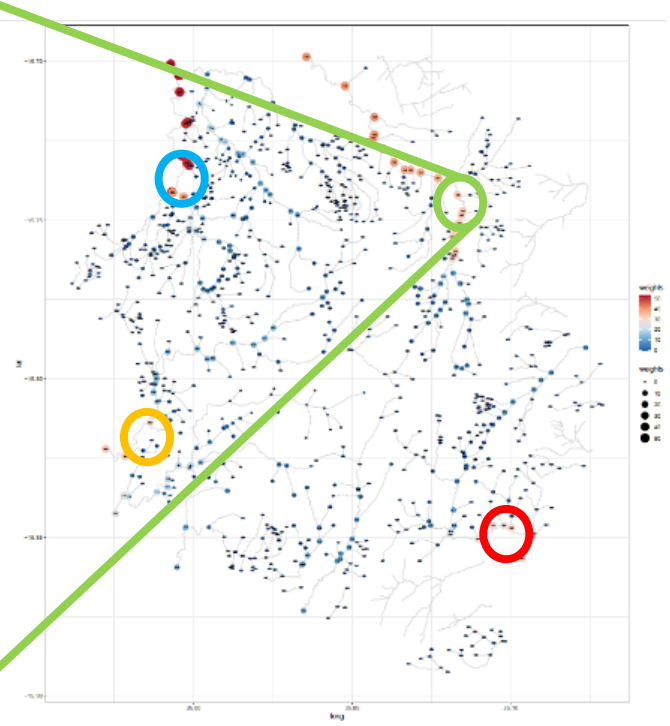


Figure 13: Examples of water access on the Lunzu River

Thuchila River

Figure 14: Examples of water access on the Thuchila River

knowledgements

Supervisors:
Dr Nicholas Feasey, LSTM
Dr Nicola Elviss, PHE
Dr Adam Roberts, LSTM

Collaborators:
Dr Satheesh Nair, PHE
Jillian Gauld, University of Lancaster
Dr Thomas Edwards, LSTM
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Dr Hywel Morgan, University of Southampton
Dr Marie Chattaway, PHE