Comparison of traditional diarrhoea measurement methods with microbiological indicators: Protocol

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Executive Summary

In slums and informal settlements, 16% of all infant deaths are attributable to diarrhoeal disease, an entirely preventable cause of death. This burden of diarrhoeal disease is due in large part to lack of access to water, sanitation, and hygiene (WASH) systems that can prevent the spread of faecal pathogens. A key challenge for WASH provision is the difficulty in evaluating and monitoring the effectiveness of WASH systems. Evaluations of WASH interventions typically use diarrhoea incidence as a primary endpoint, measured by self-reported diarrhoea and often in under-fives, which serves as a proxy for enteric infection. However, there is a notable lack of evidence on how accurate self-reported diarrhoea incidence is as a marker for enteric infection. Self-report may be subject to recall and ascertainment biases, along with the act of being observed or subject to intervention possibly leading to differential reporting.

This study will investigate the 'diagnostic accuracy' of diarrhoea self-report for enteric infection by evaluating against the sampling of stool for analysis of faecal pathogens and biomarkers of infection. Not only does this provide a means to estimate the association between infection and reported diarrhoea, but it also allows for measurement of asymptomatic infection – which is still a target of WASH interventions. This study will randomise participants into one of two diarrhoea measurement arms for surveys: a basic survey for infant diarrhoea (similar to DHS) and an enhanced survey for infant diarrhoea (using tools such as the Bristol stool scale); and subsequently randomly selecting a subgroup from each arm to undergo faecal stool testing of infants. The primary outcomes will be the positive and negative predictive values of self-reported diarrhoea for enteric infection. As a secondary outcome we will examine the types of pathogen present. We would conduct a survey at baseline in February/March, a time with low diarrhoeal disease incidence; and June/July, a time with high diarrhoeal disease incidence - to account for seasonality, and to measure variation in respondent fatigue for the various methods. This research will take place in the Cox's Bazar refugee camp, which may also allow for analysis of the burden of carriage of disease which is not endemic to Bangladesh.

Background

Diarrhoea is one of the two most common causes of death and morbidity in children under five (Kaseebaum, 2016) (Lilford et al, 2017). Upgrades to water and sanitation facilities are a key strategy to deal with this problem, for which proper monitoring and evaluation is needed to determine which interventions are working for a context, and which are not. A primary endpoint for determining the effectiveness of water and sanitation of infections is diarrhoea prevalence, both a primary endpoint in itself, in that diarrhoea is a substantial cause of morbidity and mortality in infants residing in areas with poor access to water and sanitation services; and a proxy marker for enteric infection, which in itself is may be flawed due to possibly high rates of asymptomatic carriage. As we describe, however, there are substantial concerns over the measurement of diarrhoea. We hypothesise that measurement issues may have contributed to the variable and often unexpectedly null results of recent studies, especially cluster trials, of various interventions, and are

conducting this study to determine the effect of these biases (Luby et al, 2018) (Null et al, 2018).

The presence of diarrhoea is generally assessed by questionnaire, asking whether a child has experienced three or more loose or liquid stools in a given period, typically in the past 24 hours or two weeks. However, despite the importance of this, there are few studies on the validity of this tool - with the few studies that have been conducted suggesting that current methods are not performing well (Schmidt et al, 2011). Longer recall periods, such as two weeks, are subject to fallible memory and recall bias. Shorter periods are additionally affected by other biases. For example, the more frequently a person is asked about diarrhoea, the less likely they are to report diarrhoea (Clasen et al, 2014). It has also been found that diarrhoea rates are subject to Hawthorne effects, differential behaviour due to knowledge of being observed (Leonard et al, 2006). Additionally, while loose and liquid stool are generally defined by taking the shape a container, judgement on the specifics of this may also vary between individuals. These biases may be reduced by a more comprehensive survey including pictures, such as the Bristol Stool Chart (Lewis et al, 1997). Likewise, stunting, the other outcome often used in studies of WASH interventions, is a commonly used outcome measure in studies of gastrointestinal health, however the multiple causes of growth retardation and time required for it to manifest mean it is unlikely to be sensitive to intervention in anything but the longest duration studies.

Aims and Objectives

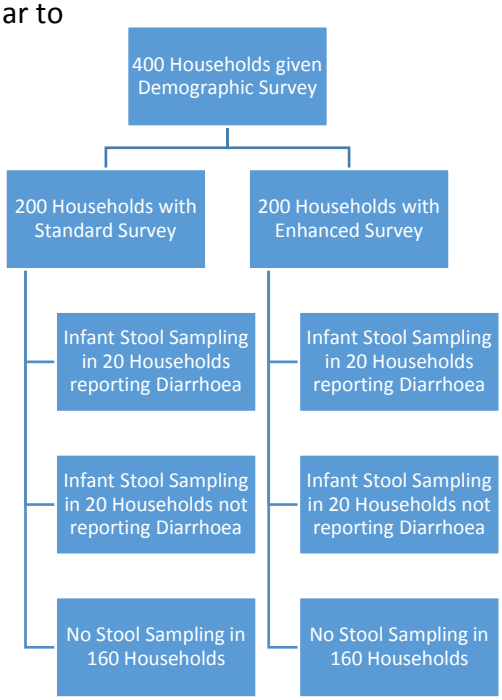
Despite the importance of the topic there have been few studies on the accuracy and reliability of diarrhoea measurement methods. The aim of this study it to evaluate the accuracy of reported diarrhoea rates as a proxy for enteric infection, using both a standard and enhanced reporting method for diarrhoea, with sub-aims:

- To assess the relative differences between “standard” and “enhanced” questionnaires.
- To analyse the pathogenic profile of stool samples.
- Determining carriage of pathogens from Myanmar to Bangladesh.

Design and Methodology

Sampling

The study will follow a two arm RCT design in which 400 households will be recruited through random household sampling in both the formal and informal refugee camps in Cox’s Bazar (Fig.1). The random household sampling will use an EPI sampling method, in which a series of random angles and distances from a pre-determined central point (e.g. a water tap) will be generated, selecting households closest to the mapped location. This list will be generated at the beginning of the data collection period, with households randomly assigned to each research assistant.



Inclusion and exclusion criteria

Inclusion and exclusion criteria for the study is as follows:

Inclusion	**Exclusion**
Household has at least one child under the age of five	Household is expecting to relocate/resettle/repatriate in the next 6 months
Household has at least one adult over the age of 18	
Household consents to study	

Recruitment

Households will be initially contacted by research assistants travelling door to door who will ask to speak with an adult in the household. The study will be explained through the use of the participant information sheet, with households meeting inclusion criteria being consented and enrolled in the study. As part of consenting, GPS coordinates, name, and mobile phone number will be recorded. Households who do not consent will not have mobile phone numbers recorded, but will have GPS coordinates recorded to ensure that the house is not approached again. Mobile phone numbers and GPS coordinates will be recorded separately from data in a log book, and linked to individual household identifiers which will be recorded on the survey form.

Diarrhoea survey

Households will be randomised in a 1:1 ratio into the basic survey, a survey asking if a child had diarrhoea and dysentery in the past two weeks, based on DHS survey measures; and the enhanced survey, based on qualified stool charts (of which images will be displayed on the tablet during surveying), proxies for diarrhoea (such as oral intake), and measures of water-washed diseases. All households will also be administered a basic demographic survey with questions on education, health, living environment, and WASH usage. If a household reports dysentery, they will be given the information of a local clinic for treatment. All surveys will be translated into Rohingya, and back translated into English – with translation following WHO guidelines: aiming for the simplest translation with the conceptual equivalent, targeted toward the target audience or Rohingya refugees, many of whom are illiterate with a low level of education. This will be done through consultation with Rohingya community members to ensure proper translation.

Data collection will take place at two time points: February/March and June/July. Research assistants will collect data through the use of ODK on an Android tablet. Data will be stored on the tablets and uploaded to Warwick servers at the end of each day, when research assistants return to the base office. Data will be additionally verified daily.

Stool sampling

Within each arm at each time point, 20 households reporting diarrhoea, and 20 not reporting diarrhoea, will be selected to have the oldest infant under 5 undergo stool testing – a total of 80 households at each time point, 160 in total. The first 20 households reporting diarrhoea and not reporting diarrhoea in each arm will be selected and asked if they are willing to provide stool samples. A WhatsApp group will be created for the research assistants to determine when 20 consenting households have been reached in each arm. The consenting households will be given a stool collection container once the survey has been administered, which will be collected the following day. All stool containers will be labelled with a barcode which will be scanned by the research assistant using a tablet, to link the stools sample with the survey. Stool will be collected by the carer, and tested for the presence of predetermined organisms which are endemic to the Rohingya population, along with a small number of select pathogens that are not endemic (Table 1); and for the presence of biological markers of infection: calprotectin and lactoferrin. This will be done through the use of polymerase chain reaction analysis (PCR) at the laboratory at the ICDDR,B in Dhaka, Bangladesh. Stool will also be tested for blood using a faecal hemooccult test.

Type	Species	Carriage period (adults)	Carriage period (children)
Bacteria	Shiga toxin-producing Escherichia coli	17-18 days (1)	35-69 days (2)
	Enteropathogenic Escherichia coli	ND	20—36 days (2)
	Enterotoxigenic Escherichia Coli	ND	<5 days (2)
	Enteroinvasive Escherichia Coli	ND	ND
	Enterohemorrhagic Escherichia Coli	ND	2-62 days (2)
	Salmonella typhi	12-25 days (3)	Varies (2)
	Salmonella paratyphi	28-55 days (4)	~30 days (2)
	Campylobacter	14-21 days (5)	Up to 6 weeks (5)
	Shigella	~28 days (6)	1-10 days (6)
	Cholera	~2 days (7)	~4 days (7)
	C. difficile	6-57 days (8)	6-57 days (8)
	C. perfringens	ND	ND
	Acinetobacter	7-11 days (9)	ND
Protozoa	Entamoeba histolytica	ND	ND
	Cryptosporidium	~8 days (10)	~8 days (10)
	Cyclospora	5-13 days (11)	22-23 days (11)
	Giardia	2-6 weeks (12)	~6 months (13)
Helminth	Ancylostoma duodenale	1-2 years (14)	ND
	Ascaris lumbricoides	Years (15)	Years (15)
Viruses	Rota-virus	Up to 35 days (16)	4-57 days (2)
	Hepatitis A	~21 days (17)	Up to 21 days (12)
	Hepatitis E	3-4 weeks (18)	ND
	Norovirus	ND	8-60 days (19)
	Human Caliciviruses	~14 days (20)	~12 days (20)
	Adenovirus	ND	8-23 days (20)
	Astrovirus	ND	1-10 days (20)
Fungi	Enterocytozoon	18-38 days (21)	9-40 days (22)

Table 1: Organisms for stool testing

Informed consent

All respondents will be consented in the Rohingya language and given a demographic and behaviour survey at the first time point. All questionnaires and surveys will be translated into Rohingya and back translated into English, with all data collection will be completed by Rohingya research assistants.

Data analysis

Data will be analysed using Stata version 15. The following analyses will be conducted

- Incidence rate ratios and associated 95% confidence intervals comparing diarrhoea and dysentery incidence between:
    - Enhanced and basic surveys
    - Survey method and stool analysis method
- Positive predictive value and associated 95% confidence interval of reported diarrhoea for the presence of enteric pathogens
- The association between diarrhoea presence and other potential markers of enteric infection such as MUAC and water-related diseases, measured for example by Jaccard's index
- A logistic regression of diarrhoea prevalence on demographic and health variables to examine association.

All missing data will be reported and assumed to be missing at random.
The sample sizes proposed here would give us power to estimate the self-reported diarrhoea rate in each arm to a 95% confidence interval of approximately ±4 percentage points, assuming a point prevalence of 10%. We would also be able to estimate the positive and negative predictive values to approximately ±7 percentage points assuming values of 90%.

Data Management

This study will produce survey data from the diarrhoea prevalence survey and results from labs studies of stool samples. Field survey data will be collected by tablet device using ODK. These data will contain identifiable information about the participants and so will be classified as 'reserved'. We will use survey software approved by the University of Warwick that abides by GCRF standards. The tablet devices will be locked by code and the field workers will have responsibility for managing the device. Completed survey forms are automatically encrypted and no longer accessible from the tablet, they are uploaded to the server and deleted when a connection is available. Lab results will be collated digitally and linked to survey participants by a unique identifier – which are also considered 'reserved'. They will be incorporated into a primary database. Data will be reviewed daily for quality. All data will be encrypted using AES-256 encryption and stored on University of Warwick servers. The data management plan for this project follows the data management plan for the NIHR health in slums project.
The data will be fully anonymised and made available publicly along with the publication planned for this work.

Ethics and Consent

Ethical approval will be obtained from the University of Warwick and a local Bangladeshi ethics board. Rohingya community groups will also be consulted in survey design to ensure that no social harm is caused.

Written consent will be obtained from the adult in the household being interviewed using an informed consent sheet in Rohingya, administered by Rohingya research assistants. The assistants will read the form to participants in the case of illiteracy. Participants have the option to withdraw participation at any time

Compensation

Compensation will not be offered as this is not anticipated to place a large time burden on participants.

Appendix 1: Gantt Framework

	November				December				January				February				March				April				May				June				July				August			
	Q1	Q2	Q3	Q4	Q1	Q2	Q3	Q4	Q1	Q2	Q3	Q4	Q1	Q2	Q3	Q4	Q1	Q2	Q3	Q4	Q1	Q2	Q3	Q4	Q1	Q2	Q3	Q4	Q1	Q2	Q3	Q4	Q1	Q2	Q3	Q4	Q1	Q2	Q3	Q4
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Appendix 2: Budget

- Laboratory work: 129 USD per test X 120 = 15,480 USD (12,090 GBP)
- Faecal occult tests: 50 GBP for 100
- Research Assistants: 60 GBP per week X 6 weeks X 4 RAs = 1,440 GBP (salary based on interpreter salary from NGOs in Cox's Bazar)
- RA local travel: 4 RAs X 28 days X 2 GBP per day = 230
- Equipment: 100 GBP Lenovo Tab8 Tablet X5 = 500GBP
- Travel for Ryan Rego: 2870 GBP
- Two surveillance visits for icddr,b: 1094 USD = 855GBP
- Stool Sample Shipping: 12USD * 2 shipments = 24USD = 19GBP
- Icddr,b admin support fee: 1950 GBP

Appendix 3: Sample Survey

Data Collection Log Book (Separated Data)

Name _____

Phone Number _____

Household Barcode SCAN BARCODE

Survey Data (Separate from Log Book)

RA	1	2	3	4

EA _____

Date						

Household Barcode SCAN BARCODE

Resident Information (all respondents)

Name	Age	Sex	Education Level	Occupation	Health Conditions	Time Spent in Bangladesh	Relationship to Respondent
RESPONDANT							
SPOUSE IF PRESENT							
Child/Other Family							
Child/Other Family							
Child/Other Family							

Child/Other Family							
Child/Other Family							
Child/Other Family							

Household Information (all respondents)

What is the main source of drinking water for your household?	Piped water into home	Piped water into plot	Piped water to tap	Borehole	Dug well	Spring	Rain	Truck	Cart	Surface Water	Bottled Water
Where is that water source located	In my home	In my plot	In a public space								
How long does it take you to get there?		Minutes									
Do you share this with other households?	Yes	No									
	If yes, how many		Households								
	If yes, how long do you have to wait for water		Minutes								

Do you do anything to make this water safe to drink?	Yes	No							
	If yes, what?	Boil	Bleach/Chlorine	Cloth Strain	Filter	Solar disinfection	Sit and Settle	Other:	
Can I see where you use the toilet?	Yes	No							
	If yes, describe:	Flush toilet to sewer	Pit Latrine	VIP Latrine	Flush toilet to Latrine	Composting Toilet	Bucket Toilet	Open Defecation	Other:
How many people use this toilet	People								
Is this toilet separated by sex	Yes	No							
Do you feel safe using this toilet during the day?	Yes	No							
Do you feel safe using this toilet when dark?	Yes	No							
How do you wash your hands?	Water alone	Water with Soap	Alcohol Based Sanitizer	Non-Alcohol Based Sanitizer	Does not wash hands	Other:			
When do you wash your hands? (all that apply)	After Defecation	After cleaning a child's stool	Before Feeding a Child	Before Eating	Before preparing food	Other:			

Do you own livestock?	Yes		No							
Where do you normally seek healthcare?										
OBSERVE MATERIAL OF FLOOR	Natural Floor	Wood	Bamboo	Tarp	Finished Floor	Other:				
OBSERVE MATERIAL OF ROOF	No roof	Thatch	Bamboo	Wood	Cardboard	Tin	Ceramic	Cement	Tent	Other:
OBSERVE WATER STORAGE VESSEL	Open Bucket	Closed bucket	Bottle	Jerry Can	Bucket	Other:				

Basic Survey (50% of respondants)

IDENTIFY OLDEST CHILD UNDER 5

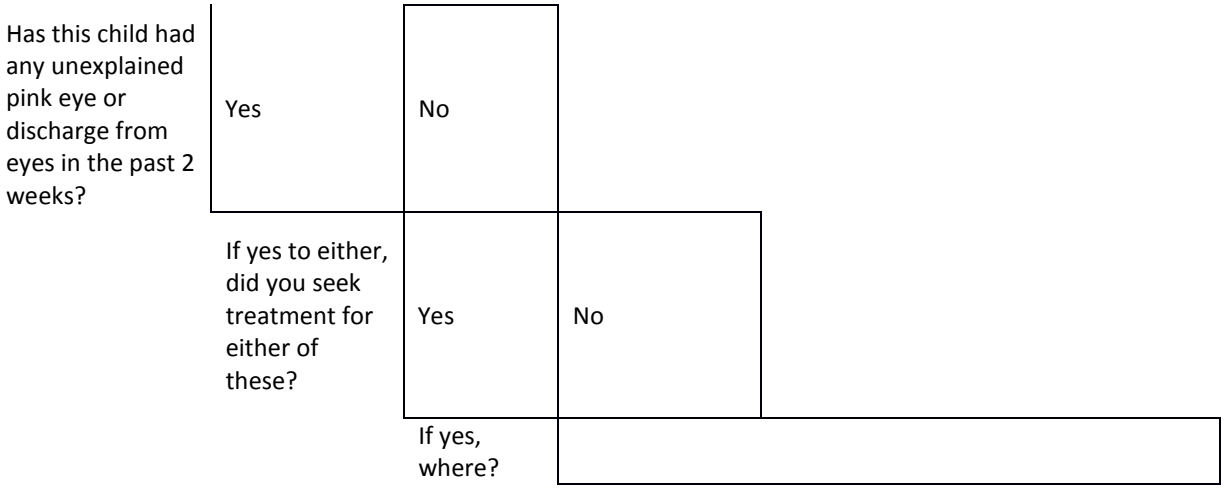
Has this child had 3 or more loose or watery stools any day in the past 2 weeks?	Yes	No
Has this child had blood in their stool in the past 2 weeks?	Yes	No

If yes to either, did you seek treatment for either of these?	Yes	No
	If yes, where?	

Enhanced Survey (50% of respondants)

IDENTIFY OLDEST CHILD UNDER 5								
Mid-Upper Arm Circumference	cm							
*SHOW AMSTERDAM STOOL CHART*Has the child had any of these stools in the past 2 weeks	Consistency	A	B	C	D			
	Colour	1	2	3	4	5	6	
How long ago?	1	2	3	4	5	6	7	Other:
How many times on the worst day?	1	2	3	4	5	6	7	Other:
How many days did it last?	1	2	3	4	5	6	7	Other:

Did this child have fever in the past 2 weeks?	Yes	No					
Has this child had blood in their stool in the past 2 weeks?	Yes	No					
Did this child have vomiting in the past 2 weeks	Yes	No					
Has this child been eating properly in the past 2 weeks	Yes	No					
	If yes to either, did you seek treatment for either of these?	Yes	No				
		If yes, where?					
	If yes to either to these, did you provide treatment?	Yes	No				
		If yes, what?	Commercial ORS	Homemade ORS	Zinc	Traditional medicine	Antibiotics	Other:
Has this child had any unexplained rashes or redness in the past 2 weeks?	Yes	No					


Has this child had any unexplained pink eye or discharge from eyes in the past 2 weeks?
Yes
No
If yes to either, did you seek treatment for either of these?
Yes
No
If yes, where?

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