

# **A Comprehensive Plan on Rotavirus Gastroenteritis Surveillance for < 5 years of age**

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## Abbreviation

BDSIS	Bhutan Diarrheal Surveillance and Information System
EIA	Enzyme Immuno-assay
EIDL	Enteric and Invasive Disease Laboratory
EQA	External Quality Assurance
PCR	Polymerase Chain Reaction
QC	Quality Control
RCDC	Royal Center for Disease Control
RT-PCR	Reverse-Transcriptase Polymerase Chain Reaction
RV	Rotavirus
WHO	World Health Organization
GRLN	Global Rotavirus Laboratory Network

## Background

Rotavirus remains the most common cause of severe, dehydrating diarrhea among infants and young children globally. Even in countries with good sanitation and hygiene, and where other causes of diarrhea may be infrequent, rotavirus infections occur frequently. Responsible for more than 120,000 of deaths and approximately 111 million episodes of gastroenteritis each year, rotavirus also necessitates more than 25 million clinic visits and 2 million hospitalizations each year. In temperate climates, every year during winter months, rotavirus diarrhea occurs in outbreaks and also prevalent throughout the year.

In 2020, Bhutan reported 3535 and 536 diarrheal diseases in < 5 years of age treated at the out-patient and in-patient department respectively with 2 mortality among the cases (Annual Health Bulletin 2020). Most of the deaths and severe diseases occur in low-income countries. Introduction of the rotavirus vaccine in a national immunization program is considered to be the most effective intervention in preventing severe rotavirus disease especially in low and middle income countries with diarrhea still the leading disease in children. Rotavirus vaccine introduction has decreased severe rotavirus gastroenteritis burden in many countries and rotavirus-associated mortality in several settings (WHO). Ideally, sites would undertake surveillance for two full years prior to vaccine introduction to assess annual and seasonal variations in disease burden, identify rotavirus genotypes circulating before vaccine introduction, and establish a stable baseline for post-vaccine introduction impact evaluations.

The Bhutan Diarrheal Surveillance and Information System (BDSIS) in Bhutan was initiated in 2015 with 12 sentinel sites participating for the sentinel diarrheal surveillance on enteric pathogens, especially focusing more on the enteric bacterial pathogens. The system captures data on the enteric viruses and EIA (Enzyme Immuno-assay) test results of the received samples, however, with no technology and logistics available to perform genotyping confirmation, there is no data to determine the rotavirus disease burden and the prevalence of circulating rotavirus genotypes in the country.

## Objectives

The objectives of the rotavirus sentinel surveillance are as follows:

1. To determine the burden of rotavirus gastroenteritis and to describe the epidemiology of rotavirus disease
2. To describe the prevalence of circulating rotavirus genotypes
3. Generate information to facilitate and support the introduction of rotavirus vaccine.

## Method

An active, case-based surveillance at sentinel hospitals with laboratory confirmation. The surveillance sites should actively look for cases in their facilities, identify acute gastroenteritis cases among children less than 5 years of age and collect data on individual cases. For all children enrolled at sentinel sites, case reporting forms are completed and a stool specimen obtained for further analysis. The forms and specimens are sent to the Enteric and Invasive Disease Laboratory (EIDL), Royal Center for Disease Control (RCDC) for data entry, analysis, testing and storage. Sample collection and processing on site for shipment to RCDC is described in SOP (**Annexure I**).

## Site selection

The sentinel sites were selected that had previously conducted diarrheal surveillance under the BDSIS in RCDC (Fig 1).

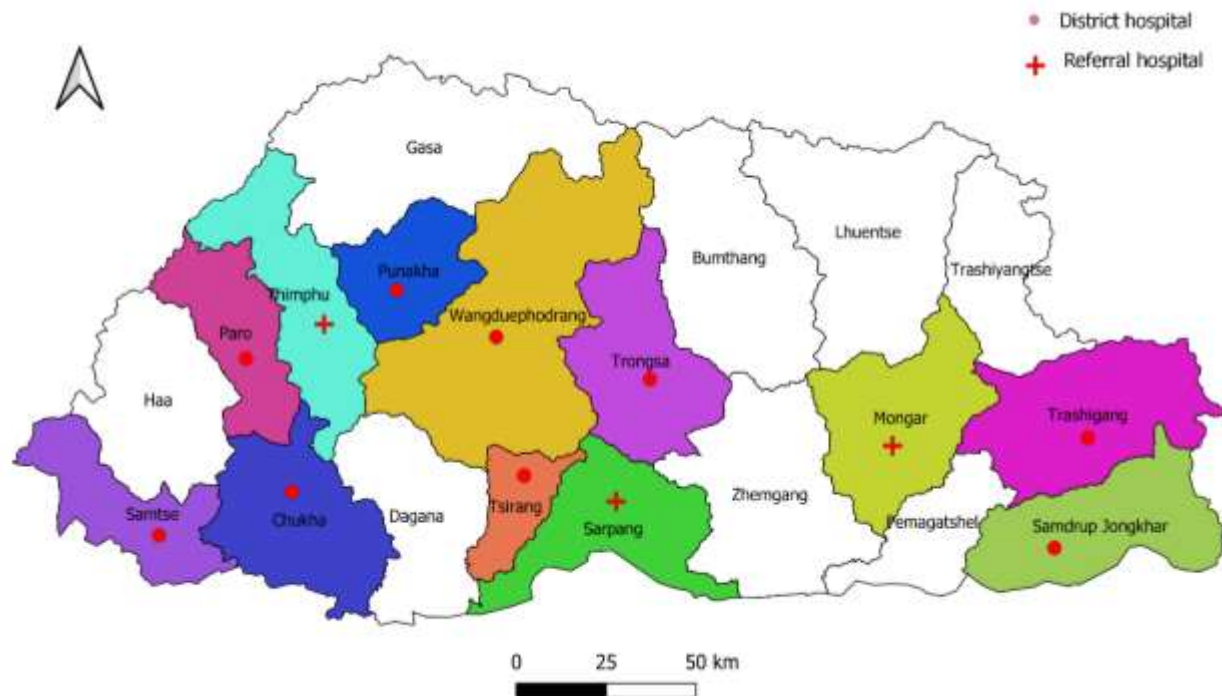


Fig 1. Rotavirus surveillance sites

## Case selection

### Suspected case definition (WHO)

Acute (<14 days) watery diarrhea, defined as three or more loose or watery stools in a 24-hour period in a child <5 years of age who is admitted for treatment of diarrhea to a hospital ward or emergency unit at a participating surveillance facility.

### Confirmed case definition:

A suspect case in whose stool the presence of rotavirus is demonstrated by means of an enzyme immunoassay (EIA) or polymerase chain reaction (PCR)-based methods

### Special Consideration

To identify for other enteric pathogens under the BDSIS, some components of the suspect and confirmed case definition might change. For example, bloody diarrhea might be included.

## Case selection and investigation

Sentinel surveillance hospitals should have a nodal person (Physicians/Pediatrician/Nurse/laboratory) designated for the surveillance. The surveillance staff in sentinel hospitals screen cases of diarrhea and identify those meeting suspect case criteria. The staff should complete case investigation forms for all cases meeting the suspect case definition. Suspect case should have a stool specimen collected within 48 hours of admission to avoid detection of nosocomial acquired pathogens.

## Sample size

To monitor the trend, continuous enrolment of children less than 5 years of age presenting to the sentinel hospitals should collect cases of diarrhea. Samples will be collected throughout the year to capture as many cases as possible, and to understand the seasonality of diarrhoeal diseases.

## Specimen Collection

Collect around 4-5 mL of stool, preferably on the day of presentation at the hospital or within 48 hours of hospital admission to avoid detection of nosocomial acquired infections. Avoid using rectal swabs or swabs placed in bacterial media. The stool specimens should be placed in sterile screw-cap containers, properly labeled.

## Sample transportation and storage

**Temporary storage:** Samples can be stored temporarily at 4 – 8 ° C for up to 1 month. However, if a specimen needs to be stored temporarily before being placed in a freezer, they should be kept at 2–8 ° C for 1 week.

**Long-term storage:** It is recommended to store stool samples that require storage over 1 month necessarily at a temperature of –20 ° C. But, care should be taken to avoid freeze thaw cycles.

**Prolonged storage:** The ability to characterize rotavirus declines, when fecal samples are stored at  $-20^{\circ}\text{C}$  for years. Therefore, when there is a need for prolonged storage, they should be kept in  $-80^{\circ}\text{C}$  freezers.

## Laboratory testing

### Detection of rotavirus

EIAs are most commonly used for rotavirus detection in stool. Several EIA kits such as: Premier™ Rotaclone®, ProSpecT™ and RIDASCREEN® will be used for the detection of the rotavirus.

### RNA Extraction, RT-PCR and Genotype-specific PCR

All positive EIA will be confirmed for the presence of the VP6 gene using RT-PCR or NP6 and NSP3 genes by real-time reverse transcription PCR (RT-PCR). The rotavirus strain characterization is one by using RT-PCR to identify both G and P types. Viral RNA was extracted using the QIAextractor (Qiagen, Hilden, Germany/ NucliSENS® EasyMAG™, Biomerieux, Marcy L'Etoile, France). All non-typeable isolates will be sent to an appropriate reference laboratory for sequencing.

## Laboratory Quality Control and Assessment

Rotavirus-positive and rotavirus-negative proficiency panel samples will be obtained from the global or regional rotavirus laboratories and assess the laboratory for competency. EIDL lab will also arrange to send some rotavirus-positive stool specimens to a regional laboratory for independent confirmation of results. External Quality Assessment (EQA) and Quality Control (QC) of the laboratory should be completed annually. The lab will also partake in the Global Rotavirus Laboratory Network (GRLN) for standardization of data collection and laboratory quality and control through a global external quality assessment program coordinated by WHO.

## Data flow

Stool samples and completed case report forms are sent to the RCDC for testing, data entry and storage. On receipt of samples and case report forms, the shipment is checked and logged, and any issues with quantity or quality recorded. Case report forms are reviewed before data entry



and data clarification in case of any missing fields or errors and sent to sites within 3 days of receipt for clarification and responses. At the EIDL, stool samples are tested for rotavirus antigen and genotyped confirmed using commercially available kits. Rotavirus test results are reported to the sites within 1 month of receiving the samples and case forms.

#### Surveillance monitoring and supervision

For the first 3 months after initiation of surveillance, sites are visited monthly. After 3 months, monitoring is reduced to quarterly in a year. At each visit, site evaluation is conducted using a monitoring checklist, which records performance in terms of enrolment of children with diarrhea and collection of adequate stool samples from all enrolled children. Every 6 months, a collaborators' meeting is organized to discuss the work done by each site and to enable collaborators to provide feedback to individual sites. Periodic review of the surveillance data collected shall identify problems in data collection, case enrollment or specimen collection and handling.

#### Data Analysis

The data shall be entered in EpiData version **3.1** and analyzed using STATA version **17**.

#### Feedback

A monthly report with the surveillance data should be prepared. This should be sent to the hospital director and the surveillance staff. In addition, quarterly surveillance meetings shall be conducted to review surveillance performance and fix key issues.

#### Reference

1. WHO, Vaccine-Preventable Diseases, Surveillance Standards, last updated: September 5, 2018.  
[https://www.who.int/immunization/monitoring\\_surveillance/burden/vpd/WHO\\_SurveillanceVaccinePreventable\\_19\\_Rotavirus\\_R2.pdf](https://www.who.int/immunization/monitoring_surveillance/burden/vpd/WHO_SurveillanceVaccinePreventable_19_Rotavirus_R2.pdf)
2. Nayana P Nair et al., 2018, Rotavirus vaccine impact assessment surveillance in India: protocol and methods, <http://dx.doi.org/10.1136/bmjopen-2018-024840>
3. Arif Mohammad Tanmoy et al., April 20, 2016, Rotavirus Surveillance at a WHO-Coordinated Invasive Bacterial Disease Surveillance Site in Bangladesh: A Feasibility Study to Integrate Two Surveillance Systems, Published: April 20, 2016,  
<https://doi.org/10.1371/journal.pone.0153582>
4. WHO, Rotavirus Gastroenteritis, Surveillance Guide for Vaccine-Preventable Diseases in the WHO South-East Asia Region, Module-8, Regional Office for South-East Asia

## **Annexure- I: Sample Collection and Processing**

**At Sentinel site:** The microscopic examination, culture and AST will be performed for the collected specimen wherever there is a microbiologic facility. Again, the stool specimen will be aliquoted in cryo-vials along with patient details and stored at -20°C prior to shipment. Approximately one gram of stool will be saved in properly labeled Cary-Blair transport media and stored at room temperature prior to shipment. Any isolated organism will be subcultured in N/A slant and shipped to RCDC along with the Cary-Blair saved and frozen specimens.

**At Enteric and Invasive Disease Laboratory (EIDL), RCDC:** Microbial culture will be repeated to isolate all kinds of enteric pathogen (i.e. *E.coli*, *Salmonella*, *Shigella*, *Aeromonas*, *Plesiomonas*, and *Campylobacter*) and perform AST for the isolated organism. For all isolated *E.coli*, multiplex PCR will be performed to identify diarrheagenic *E.coli* which includes ETEC, STEC, EPEC, EAEC and AST will be performed on all positive strains. ELISA will be performed on all frozen specimens to detect rotavirus, astrovirus, adenovirus, norovirus and combined giardia/cryptosporidium. Any suspicious isolates will be sent to WHO-collaborated laboratories for confirmation from RCDC. The remnant frozen specimens will be stored at -80°C for future reference.

## Annexure-II: Sentinel Surveillance for Diarrhea Etiologic Agents Case Investigation Form

Lab ID:

Date of hospital visit:

DEMOGRAPHY		
Name:	Age:	
Gender: Male <input type="checkbox"/> Female <input type="checkbox"/>	Occupation:	
Residential address:	District:	
Contact Number:		
OPD/IPD:	Category: Surveillance <input type="checkbox"/> Outbreak <input type="checkbox"/>	
Date of onset of illness:		
CLINICAL INFORMATION		
Syndrome	Yes	No
Watery diarrhea		
Bloody diarrhea		
Loose diarrhea		
Persistent diarrhea (last 2-4 weeks)		
Chronic diarrhea (last more than 4 weeks)		
Suspected foods/drinks consumed in the past 72 hours: Yes <input type="checkbox"/> No <input type="checkbox"/>		
If yes, specify (name the food/drinks):		
Travel history: Yes <input type="checkbox"/> No <input type="checkbox"/>		
If Yes, Place name:	Date :	
LABORATORY RESULT		

**Stool R/E:**

Color: .....

Consistency: Watery  Loose  Formed

Mucus seen: Yes  No

Blood seen: Yes  No

RBCs..... /HPF

WBCs..... /HPF

Ova/cyst.....

Others.....

Culture and AST if performed:

Sample aliquot in cryo tube?: Yes  No

Date of shipment to RCDC:

Specimen processed by (Name of Lab personnel):