

National Diarrheal Disease Surveillance System

Background

Diarrhoeal disease is a public health problem, especially in countries where poverty prevails and is the leading cause of child mortality and morbidity in the world, and mostly results from contaminated food and water sources. Diarrhoeal disease is the third leading cause of death in children under 5 years old and is responsible for killing around 443,832 children every year [1], with attack rates ranging from two to 12 or more illnesses per person per year in developed and developing countries. Worldwide, 780 million individuals lack access to improved drinking water and 2.5 billion lack improved sanitation. Diarrhoea due to infection is widespread throughout developing countries. In recent decades, deaths from diarrheal diseases have fallen significantly across the world, as a result of public health interventions. But more progress is possible. Diarrheal deaths are preventable because they are primarily caused by pathogens, whose spread can be easily controlled. By increasing global access to clean water and sanitation, oral rehydration treatment, and vaccination, this major cause of death can be reduced [2]

The most common cause of diarrheal disease results from gastrointestinal infections. Gastrointestinal infections are a common global health problem. The cause of diarrhea includes a wide array of viruses, bacteria and parasites [3]. Known viruses causing diarrhea include adenovirus, rotavirus, astrovirus and norovirus. The common bacterium associated with diarrhea includes *Shigella*, *Salmonella*, *Aeromonas*, *Plesiomonas*, *Campylobacter* and pathogenic *E. coli*. *Giardia*, *Cryptosporidium* and *Entamoeba histolytica* are among the parasites reported to be associated with diarrhea besides other parasitic organisms like *Taenia* and others.

Although significant strides have been made in the reduction of mortality from diarrheal dehydration with the introduction of oral rehydration therapy (ORT), this intervention offers little benefit in patients with dysentery and other enteric infections. No single antibiotic behaves as a magic bullet to cure all diarrheal diseases as it all depends upon the antimicrobial susceptibility pattern of the individual pathogen. Again, due to the overuse of antibiotics in clinical settings, there has been an increasing antibiotic resistance among bacterial enteropathogens and notably observing an increase in multi-drug resistance patterns in the enteropathogens.

Bhutan has made substantial efforts in expanding water supply and sanitation infrastructure mainly with international support over the past decades. Despite increasing coverage, diarrheal diseases are reported every year. Acute watery and bloody diarrhoeas are reported under syndromic surveillance from all health centres through event-based reporting in the National Early Warning Alert and Response System (NEWARS) established by Royal Center for Disease Control (RCDC) [4]. Many similar events were unrecognized and under-reported without any diarrheal disease surveillance system in the country. Rapid detection of pathogenic organisms that cause diarrhoea is essential for the timely management of patients as well as to help identify the source of impending outbreaks. Therefore this Indicator-Based Surveillance (IBS) on diarrheal diseases will help understand the epidemiology of diarrheal

diseases in the country. The goal of the surveillance is to provide evidence-based information for controlling diarrheal disease in the country.

Objectives

1. To estimate the burden and magnitude of diarrheal disease.
2. To identify high-risk areas and high-risk groups for providing special attention.
3. To study the trend (spread and progress) of diarrheal diseases in different seasons.
4. To monitor the antimicrobial susceptibility pattern of all isolated bacterial pathogens
5. To monitor changes in pathogens causing diarrheal diseases including the emergence of new pathogens.
6. To detect outbreaks of diarrheal diseases and determine their cause and source for implementing prevention and control measures.
7. Support plan for distribution of medical supplies which includes diagnostic test and antibiotics in the health centers.

Materials and Methods

Case definition

Any patient with passage of three or more loose or watery stools in the past 24 hours with or without dehydration.

Study sites

The surveillance will be conducted in Trongsa, Phuntsholing, Samtse, Paro, S/Jongkhar, Tashigang, Mongar, Gelephu, Tsirang, Punakha and JDWNR Hospital (Fig 1). These study sites were selected based on already existing surveillance activities to reduce the cost and improve the performance of the system.

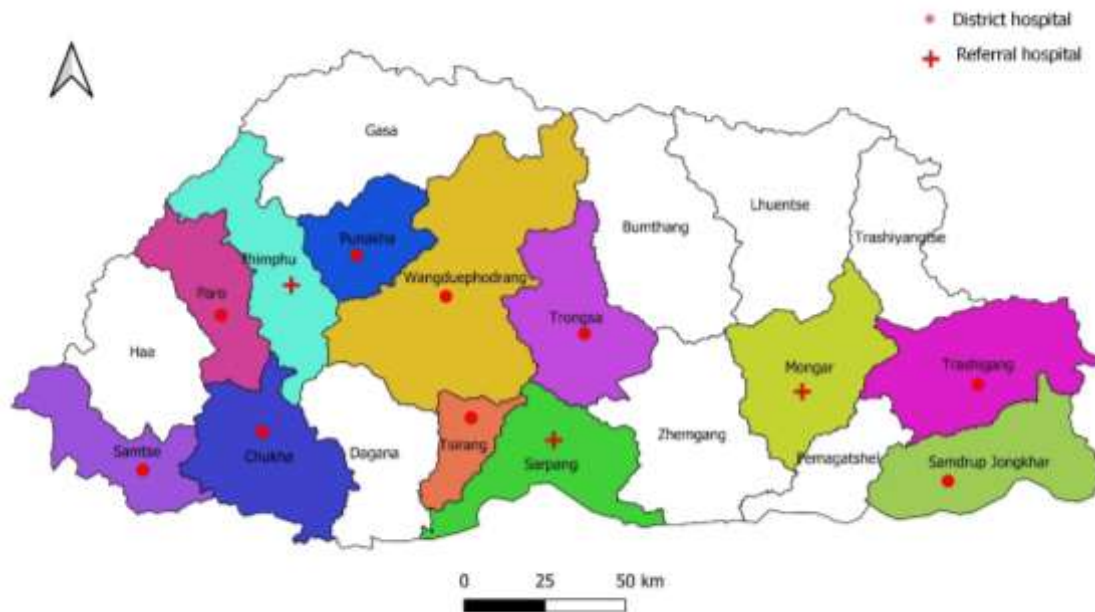


Fig 1. Sampling sites

Enrollment

All subjects meeting the case definition will be enrolled from both OPD and IPD. Three to four specimens per week from each site will be collected before the initiation of the antibiotics to improve the recovery of pathogens. Specimens can be directly passed into the sterile wide-mouthed container or collected from the napkins into stool containers for children. Specimens will then be transported to the laboratory in a cold chain within a one to two-hour period.

Data collection

After explaining and obtaining the informed consent, a unique ID will be assigned to each subject and baseline information will be collected using a standardized questionnaire (Case Investigation Form, available at https://www.rcdc.gov.bt/web/?page_id=257) followed by specimen collection. All the data regarding the sample and test results/values will be entered into a database maintained in RCDC (<https://www.rcdc.gov.bt/login.ph>). In addition, each hospital administration will appoint a dedicated surveillance focal person to report the total number of cases (both OPD and IP) as well as the number of diarrheal cases observed at each site.

Specimen collection, processing and storage

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 before shipment. Approximately one gram of stool will be saved in properly labelled modified Cary Blair

transport media (mCB) and stored at 2 to 4°C before shipment. Any isolated organism will be sub-cultured in N/A slant and shipped to RCDC.

At Enteric, Zoonotic and Vector-borne Disease Laboratory (EZVBDL), RCDC: Microbial culture will be repeated to isolate all kinds of enteric pathogens (i.e. pathogenic *E.coli*, *Salmonella*, *Shigella*, *Aeromonas*, *Plesiomonas*, *Yersinia* 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*, *EIEC*, *EAEC* and AST will be performed on all positive strains. ELISA will be performed on all frozen specimens to detect rotavirus, astrovirus, adenovirus and combined giardia/cryptosporidium. Any suspicious isolates will be sent to WHO- WHO-collaborated laboratories for confirmation from RCDC. The remnant frozen specimens will be stored at -80°C for future reference.

Antibiotic Susceptibility Testing

The antibiotic sensitivity testing of bacterial isolates was conducted using the modified Kirby-Bauer disc diffusion method, following the guidelines outlined by the Clinical and Laboratory Standards Institute (CLSI). This method is a standard procedure utilized for interpreting the susceptibility of bacterial isolates to various antibiotics based on the size of the inhibition zones surrounding antibiotic discs. CLSI recommendations ensure consistency and accuracy in interpreting susceptibility results, thereby facilitating informed decision-making regarding antibiotic therapy.

Feedback and Report

Feedback will be provided by anyone working in EZVBDL to the sentinel site and vice versa. The provision of feedback is to acknowledge as well as to inform other stakeholders of the need for improvement or to take some corrective measures.

A monthly report on diarrheal disease surveillance will be shared with the sentinel sites to ensure their participation and contribution to the surveillance efforts. The sentinel sites can also view the comprehensive report quarterly and annually at https://www.rcdc.gov.bt/web/?page_id=3561.

References

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