PROTOCOL FOR DENGUE SENTINEL SURVEILLANCE 2020

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1 BACKGROUND

1.1 GLOBAL & REGIONAL

Dengue is an emerging vector-borne disease with increasing reports of outbreaks. In 2012, WHO estimated around 50–100 million new infections annually in more than 100 endemic countries with hundreds of thousands of severe cases arising and around 20000 deaths every year. The true numbers are probably far worse, since severe underreporting and misclassification of dengue cases have been observed. During the past five decades, the incidence of dengue has increased 30 folds with a documented further spread to previously unaffected areas. Moreover, dengue outbreaks exert a huge burden on populations, health systems and economies in most tropical countries of the world. The emergence and spread of all four serotypes of dengue viruses (DENV) from Asia to the Americas, Africa and the Eastern Mediterranean regions represent a global pandemic threat. Although the full global burden of the disease is still uncertain, the patterns are alarming for both human health and the economy especially in the Asia Pacific Regions where about 70% (1.8 billion) exposed to dengue resides.

In South East Asia, dengue is of particular importance with most countries bear high burden of DF/DHF and experience frequent and cyclical epidemics. About 1.3 billion live in 10 countries of the WHO South-East Asia (SEA) Region which are dengue endemic areas. Reported case fatality rates for the region are approximately 1%, but in India, Indonesia and Myanmar, focal outbreaks away from the urban areas have reported case-fatality rates of 3--5%. Since 2000, epidemic dengue has spread to new areas and has increased in the already affected areas of the region. In 2003, eight countries including Bangladesh, India, Indonesia, Maldives, Myanmar, Sri Lanka, Thailand and Timor-Leste reported dengue cases. In 2004, Bhutan reported the country's first dengue outbreak. In November 2006, Nepal reported indigenous dengue cases for the first time. Over the past four years, epidemic dengue activity has spread to Bhutan and Nepal in the sub-Himalayan foothills.

1.2 BHUTAN

Dengue was first detected in Bhutan in 2004 with massive outbreak affecting across all age groups however, no death was reported. Dengue has now become endemic in south where dengue vectors are prevalent. During last seven-eight years, dengue annual incidence has increased almost 3 folds as per the passive reporting but the true numbers are probably far less. There is also emerging evidence of expansion of geographical area by vectors in the country. Molecular characterization of dengue virus from randomly collected acute phase blood samples from dengue suspected cases during 2004-2006 in Phuntsholing hospital found presence of serotypes DENV1, DENV2 and DENV3 with DENV2 predominated in 2004 and DENV3 in 2005-2006.

1.3 SCIENTIFIC RATIONALE

Dengue is a neglected disease in most part of the world, including Bhutan, and true burden is largely unknown. The epidemiology of DF/DHF is complex and remains poorly understood. It involves host, viral and epidemiological status which are further influenced by demographic, economic, behavioral and varied societal factors. Many field observations have raised questions against widely accepted epidemiological characteristics of dengue. It is thus imperative to properly understand the evolving pattern and trend of DF/DHF epidemiology, as it is crucial in determining the success of prevention and control programme.

In Bhutan, no systematic studies have been published till date that attempt to determine the true burden of dengue in the general population prospectively through established sentinel surveillance. Neither there is proper epidemiological surveillance system in place to demonstrate the impact of dengue. The determination of true dengue burden including the characterization of serotypes in circulation will assist clinicians and public health personnel in understanding more about the dengue disease, its management and preparing effective preventive interventions.

2 OBJECTIVES:

This surveillance aims to study the epidemiology of dengue virus infection by

- Determining the circulating serotypes of DENV in the country
- Finding out risk factors and population at high risk to DENV infection
- Establishing a national baseline and burden of DENV virus infection among Bhutanese population
- To monitor the burden of Dengue
- To characterize epidemiology and clinical features
- To monitor dengue virus serotypes/strains
- To predict and prevent outbreaks

3 SURVEILLANCE DESIGN

3.1 CASE DEFINITION

Fever (oral, rectal or axillary temperature ≥38°C) or history of fever of unknown origin with two or more of the following symptoms- headache, retro-orbital pain, myalgia (muscle pain), arthralgia (joint pain), severe back-pain, rash, haemorrhagic manifestation (petechiae and positive tourniquet test) and leucopenia.

3.2 SURVEILLANCE SITES

The following district hospitals will serve as sentinel sites for the surveillance

Sl. No.	Surveillance site	Site code
1	Phuntsholing Hospital	BTF
2	Samtse Hospital	BTK
3	Samdrup Jongkhar Hospital	BTJ
4	Central Regional Referral Hospital, Gelephu	BTG
5	Nganglam Primary Health Care Centre	BTN

3.3 CASE ENROLLMENT AND DATA COLLECTION

Cases that meet the inclusion criteria will be enrolled. Ambulatory patients will be enrolled through outpatient department (OPD) and emergency department while admitted patients will be enrolled through the in-patient department (IPD). For OPD cases, the attending clinician(s) will fill up the case investigation form (Appendix 2) and send the patient along with the form to laboratory for sample collection. For admitted and emergency cases, attending staff nurse will fill up the form, collect sample and send the sample along with the form to laboratory.

The patient will be requested to make a visit 1 week after the initial hospital visit for follow-up (convalescent) sample testing. Patient will be sent to laboratory. Laboratory technician will fill up the same case investigation form (Appendix 2) but label the sample as explained below.

3.4 CASE AND SAMPLE IDENTIFICATION

Each case/ sample will be labeled using a bar-code supplied by RCDC. The first 3 letters in the bar-code will denote the surveillance type, for instance, “DEN” for dengue, followed by site code, “BTF” for Phuntsholing, a running number for each sample collected, for instance “001” for the first sample, and finally the letter “A” or “C” to denote whether the sample is acute or convalescent. So a sample labeled as DEN-BTF-001-A will mean that this is the acute sample from the first case of dengue surveillance from Phuntsholing Hospital. The hospital will maintain a surveillance registry log sheet to keep track of cases and samples.

3.5 SPECIMEN COLLECTION AND TESTING

Laboratory technicians will collect venous blood specimens in plain and EDTA vacutainers from each case as per the surveillance plan (Appendix 1). All blood specimens will be labeled with laboratory identification number. The lab will perform a complete blood count using EDTA blood and a rapid test for dengue using serum. Serum from each case will be further stored for further shipment as given below.

Laboratory person will contact patient for collection of lab result and convince patient to provide convalescent sample.

3.6 STORAGE AND TRANSPORTATION

Serum sample will be kept on 2-8^{o}C cold chain for maximum of 7 days at sites and sent to RCDC on a weekly basis. Cold-chain should be maintained during shipment. Samples after testing in RCDC will be stored at -80^{o}C until further shipment to a designated reference laboratory.

3.7 LABORATORY DIAGNOSTICS

3.7.1 Hematology tests

Hospital laboratories will perform complete blood count (CBC) for all enrolled cases to facilitate in diagnosis and classification of dengue infection using auto-analyzer.

3.7.2 Serology tests

Acute samples will be tested for dengue virus NS1 (non structural protein of dengue virus) and IgM using ELISA test kits. Convalescent samples will be tested for IgM and IgG ELISA to diagnose and determine the proportion of primary and secondary dengue infection. The test procedures will be performed as per the package insert of the test kit available at the RCDC.

3.7.3 Polymerase Chain Reaction (PCR)

Samples that are positive by serology for NS1 and IgM, that have adequate volume will be selected for determining serotype. A nested RT-PCR will be performed using method modified from Lanciotte et al (1992). Samples for serotype analysis will be selected to represent every sentinel site and will include outbreak samples from non-sentinel sites.

3.7.4 Gene sequencing

Selected positive samples will be subjected to genetic sequencing. Whether to perform sequencing for the whole genome or partial genome (usually the envelope gene) will depend on the need of the situation. Virus culture and isolation will be performed if sequencing is not feasible from the original specimen. Selection of specimens for sequencing and isolation will depend on the number of positives and serotypes available.

3.8 DATA HANDLING

3.8.1 Disposition of Data

Source data sheets (Clinical form) and test results will be stored at RCDC. Any identifiable data will be removed and information from the clinical form will be entered into a database maintained at RCDC for analysis. CBC results of any dengue positive both by PCR and ELISA tests will be followed up.

3.8.2 Data Analysis

Data analysis will consist of descriptive statistics, standard statistical tests and genotyping using standard using standard software/tools.

3.8.3 Feedback/Reports

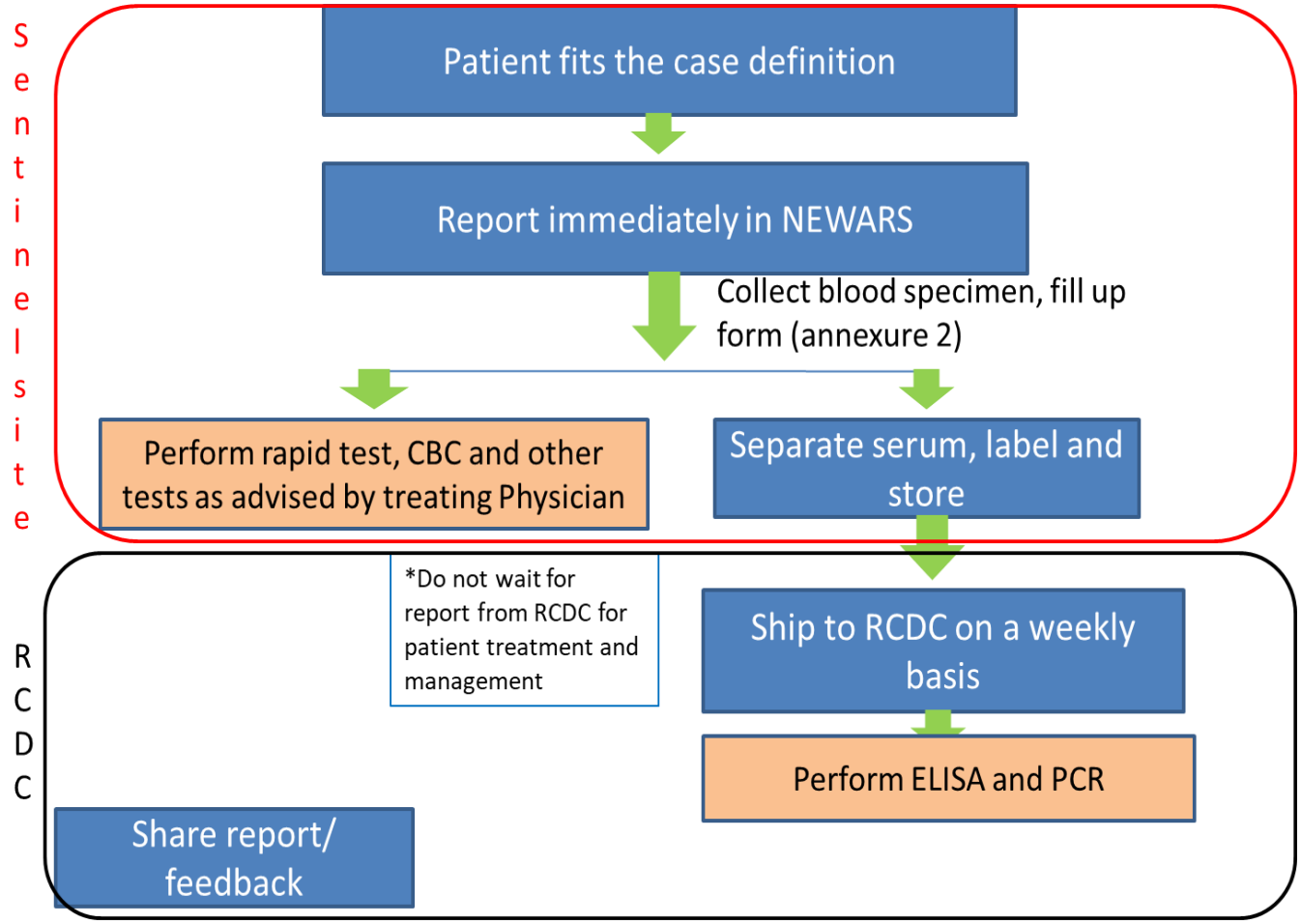
Test results will be sent on a weekly basis to the sites by case number to notify the physician/ patient' by the laboratory persons. Both preliminary and final reports of the surveillance will be made available to sites and collaborating stakeholders.

3.9 TRAINING

Personnel including nurses, laboratory technicians and clinicians working at the surveillance sites as part of the team will be trained on patient enrollment, specimen collection, storage, transportation of specimens, and universal precautions before the surveillance starts.

4 APPENDICES

4.1 APPENDIX 1. SURVEILLANCE PLAN



4.2 APPENDIX 2. CASE INVESTIGATION AND SAMPLE COLLECTION FORM

Case Investigation and Specimen Collection Form for Dengue Fever/Dengue Hemorrhagic fever (Version-1)					
Case type please tick: □ Dengue Fever □ Dengue Hemorrhagic fever					
Patient Information					
Name of Health Centre:			Date DD/MM/YY		
Patient Name:			Age/Sex:		
Contact Number:			Occupation:		
Residential Address:					
Clinical Information					
Fever	□ Yes	□ No	Retro orbital pain:	□ Yes	□ No
Joints pain	□ Yes	□ No	Myalgia	□ Yes	□ No
Backache	□ Yes	□ No	Haematemesis or melena	□ Yes	□ No
Petechaie, ecchymoses, or purpura	□ Yes	□ No	Altered mental status	□ Yes	□ No
Nausea/vomiting	□ Yes	□ No	Fatigue	□ Yes	□ No
Rashes	□ Yes	□ No	Restless	□ Yes	□ No
Chills	□ Yes	□ No	Others (Specify):		
Co morbid conditions (Check all that apply)					
□ None □ Diabetes □ Cardiac Disease □ Hypertension □ Pregnancy					
BP _____ Pulse _____ Tempt _____					
Hospitalization: □ Yes □ No			Date of Admission:		
Outcome: □ Recovered □ Referred □ Death					
Epidemiological Information					
Date of notification::_____ Date of onset of symptoms:_____					
Residing in dengue endemic places □ Yes □ No					
Have you travel to dengue endemic places: □ Yes □No If yes, place visited _____					
Travel dates: From _____ To _____					
Laboratory information					
Laboratory Specimen Collected: □ Yes □ No			Sample ID		
Lab result CBC □ Yes □ No if yes □ WBC_____ HB_____ HCT_____PLT_____					
Lab result RDT : ☐ NS1 positive ☐ IgM positive ☐ IgG positive					
Lab result ELISA: (RCDC) ☐ positive ☐ Negative:					
Trioplex RT-PCR result (RCDC) ☐ DENV positive ☐ CHICK positive ☐ ZIKAV positive _____:					
Sample Collected by: Name: _____ Contact #:					