ENHANCED SURVEILLANCE ON SEROPREVALENCE OF SARS-COV-2 IN GENERAL POPULATION OF NEPAL

Summary of the proposal

The study entitled “ENHANCED SURVEILLANCE ON SEROPREVALENCE OF SARS-COV-2 IN GENERAL POPULATION OF NEPAL” will be conducted in each of the 7 provinces of Nepal in order to estimate point prevalence of COVID-19 antibody in general population of Nepal. The study is cross sectional where multistage cluster sampling technique will be used. In total, 3150 (450 from each of province) samples will be collected from 7 provinces. Thirty clusters will be selected on probability proportionate to size sampling (PPS) basis from each of the 7 provinces. The cluster is here defined as ward in a rural or urban municipality. In case of large ward, the ward will be segmented into two or more segments, each segment having not more 200 households. One segment will be selected randomly and 15 House Holds (HH) from each of cluster will be selected using systematic random sampling technique. From the selected households, one individual will be identified with the help of Kish Grid Table. The identified individual will be interviewed and 3-5 ml whole blood from each adult in the HH will be collected. In case of children under 5 years of age, 1-2 ml blood will be taken by using Scalp vein set on to DBS filter paper. The serum will be extracted and tested with WHO validated or country of origin FDA licensed by the countries of origin ELISA kit for the presence of IgG against COVID-19. The data will be analyzed and seroprevalence in general population would be determined.
Background

Coronavirus disease 2019 (COVID-19) caused by SARS-CoV-2 has emerged as a pandemic and spread to more than 200 countries. Amongst those infected, the proportion of asymptomatic patients could be as wide as between 5 and 80 percent. We can determine the extent of infection in the general population, age-specific point prevalence and estimate the proportion of asymptomatic or subclinical infections by correlating history of symptoms, signs and other available tests, with results of serologic tests for antibodies. Cross-sectional sero-sampling investigation provides snap shot estimates of infection in the population. This study is designed to understand the extent of COVID-19 infection in the community.

COVID-19 has been reported from all 77 districts of Nepal, the number of cases and deaths are in increasing trend. This targeted seroprevalence study will help to decide upon the stage of transmission first with information on level of infection and second with information of risk of transmission in the community.

General Objective

To determine seroprevalence of SARS-CoV-2 in the general population of Nepal.

Primary specific objectives:

- To determine the seroprevalence for SARS-CoV-2 infection in each province of Nepal.
- To determine age, sex and place, comorbidity, history of travelling and symptoms specific seroprevalence, as determined by seropositivity

Secondary objective

- To determine intensity or stage of transmission which will contribute to make decision on preparedness and response to COVID-19.

Research design Cross Sectional

Description of research design

This sero-epidemiological study is an adjunct to the national surveillance program for COVID-19 and is designed as a cross-sectional cluster survey of a statistically robust random sample of the population, irrespective of age or past history of COVID-19 infection, history of travelling, symptoms and other medical conditions. The primary sampling unit or cluster will be a ward (recent) in a Palika. All the clusters as ward in a province will be listed in order. 30 wards in each province will be selected via probability proportionate sampling (PPS) as per standard WHO protocol for immunization coverage evaluation survey. In case of large ward being selected, the ward will be segmented as frames of 200 households each. One of these segments will be selected randomly for sampling the households. For this purpose, local FCHV and ward president will be consulted for support.
Study site and Justification

Selected wards of province are sampling sites. 30 wards will thus be selected as 30 clusters. The selection of sampling frame and primary sampling units as well as clusters shall be done by PPS using randomization centrally and the study partners implementing the study in the field shall be given this list. As this study will estimate provincial level seroprevalence of COVID-19, all wards that are in the sampling frame will be represented with equal probability.

Study Population

Any person, irrespective of age or history of COVID-19 infection and living in Nepal for a continuous period of at least 6 weeks prior to the first blood sampling date.

Sampling unit

Any person selected as per Kish Grid table in selected households.

Sample size

450 from each province and 3150 in total.

Number of participants and Justification

Number of participants was calculated on following assumptions:

- Only one person per household will be selected randomly to minimize intra cluster correlation.
- Nonresponse (maximum): 10%

Sampling House Holds

When an empty household is selected, the team will move to the next household and complete the survey until the required number of households are visited. When a household refuses to participate the household will be discarded and no replacement household will be selected. 10% refusal rate has been included for this occurrence. Only one person per household will be randomly selected using the Kish grid that takes into account the number of people present in the household. The sample selection by Kish Grid table shall be explained to the data collectors during training so that a predictable and auditable model for data collection is ensured. Eligible people are those who fulfil the inclusion criteria and who routinely sleep at the household even if they are absent at the time of the first visit. Eligible people will be tabulated from youngest to oldest, and the participant selected according to the household number and number of eligible participants, by Kish method. After the selection of the interviewee and obtaining informed consent, the data collector (enumerator) will complete the interview immediately as per the questionnaire or arrange an appointment at a convenient time for the selected participant to be interviewed. In case the selected participant is not present the data collector will arrange a time to find him/her.
Criteria for sample selection

Inclusion criteria:

• All individuals identified for recruitment into the investigation, and who has arrived in Nepal at least 6 weeks prior to first blood sampling date, irrespective of age or prior history of COVID-19 infection in subject or any family member or contacts.
• Suspected or confirmed prior COVID-19 infection will not be considered as an exclusion criterion for this investigation.

Exclusion criteria:

• Refusal to give informed consent, or contraindication to venipuncture.
• To allow for enough time for exposure to infection and seroconversion, any person who has arrived in the country less than 6 weeks prior to the intended blood sample collection date will be excluded.

Data Collection Technique

After obtaining written informed consent (from selected participant or parent/guardian if child), each participant/parent/guardian recruited into the investigation should be asked questions to complete a questionnaire which covers demographic, determining factors, and exposure history to laboratory-confirmed COVID-19, symptoms suggestive of COVID-19 after January 2020 and clinical history.

A blood sample needs to be collected from each participant upon recruitment into the investigation. Serum should be separated by using screw capped vials for serum separation and using a centrifuge generally available at-least at the district health facility from whole blood and can be stored and shipped at +4°C or frozen to -20°C or lower and shipped on dry ice the same day to NPHL. The collection of blood samples should follow specimen collection guidance in Nepal as followed for measles and other surveillance investigations. All those involved in the collection and transportation of specimens should be trained in safe handling practices and spill decontamination procedures. For each biological sample collected, the time of collection, the conditions for transportation and the time of arrival at the study laboratory will be recorded. Specimens should reach the laboratory as soon as possible after collection. In exceptional situations, where, the specimen is not likely to reach the laboratory within 72 hours, specimens should be frozen, preferably at -20°C, and shipped on dry ice. It is, however, important to avoid repeated freezing and thawing of specimens. The storage of serum specimens in domestic frost-free freezers should be avoided, owing to their wide temperature fluctuations. Transport of specimens should comply with applicable national regulations.

Serum samples will be tested for the presence of COVID-19 virus specific IgG antibodies using serological testing. Tests for IgG will be carried out using an enzyme linked immunosorbent assay (ELISA) in BSL2 lab at NPHL, Kathmandu.
**Team for Data Collection:**

Each team will be composed of two investigators plus one phlebotomist: The two investigators will work as data collector and local coordinator and one local lab technician or Lab Assistant will be inducted for the day from the nearest PHC or public or private laboratory (as guided by existing network for measles surveillance system). The data collector will be trained before visiting the field. S/he will be responsible for selection of segment of cluster, households and individual participant after conducting a meeting with the local ward president and the respective FCHVs. Then, the data collector will take informed consent, interview the identified individual or his/her primary care taker and fill the information in electronic version the database on a tab provided. The phlebotomist will be responsible for blood collection, coding of sample, and serum extraction. The samples will be linked to interview forms through unique IDs and sent to NPHL through pre-existing national courier system supported through WHO-NPHL arrangement from the district level. There will be one supervisor in each province who will supervise the field work, manage sample transportation and coordinate with the palika.

Appropriate PPE (gloves and masks for investigators and gloves, masks and face shield for phlebotomist) will be provided to the teams for their biosafety by EDCD/WHO. They will be trained for appropriate use and disposal of PPE.

**Data collection tools**

Structured Questionnaire provided by WHO/EDCD

**Plan for supervision and monitoring**

Supervision and monitoring will be done from Federal and Provincial Health Office (Ministry of Social Development and/or Provincial Health Directorate).

**Plan for data management and analysis**

Attempt will be made to capture all data electronically at point of data collection itself. Data will be stored securely by WHO/EDCD. The seroprevalence of SARS-CoV-2 infection will be determined. Age and gender categories prevalence will be calculated for each province and a summary estimate for the country. The association of seroprevalence with travelling history, symptoms history and comorbidity also will be determined by appropriate epidemiological measures with statistical tests of significance.

**Expected outcome of the research results**

Point prevalence of COVID-19 of each Province will be determined. Association of age, sex, place, history of travelling, history of symptoms and co-morbidities in relation to COVID-19 will be determined.

**Plan for utilization of research findings**
The data obtained from this enhanced surveillance will help Ministry of Health and Population to map the policy and strategies to cope with the trend of COVID-19. The findings will also suggest the direction of the spread of COVID-19 transmission.

**Ethical Consideration**

This study is an extended arm of the national surveillance for COVID-19. It is therefore standard public health surveillance of national importance and not research per se. Nevertheless, as a measure of abundant caution, informed consent will be sought from each participant and/or from legal guardian of each participant (if minor aged below 18 years). The investigators will explain the objectives, procedure and importance of the study. Then they will request the participants to give consent voluntarily. All the individual participants will be given unique identification number (ID). The link of ID to individuals will be maintained by the investigation team and WHO/EDCD. The investigators will be trained to maintain confidentiality. The participants will also be informed that they would get test results later through phone calls. While communicating with individuals participating in the study proper counseling will be provided.

Although this is extended surveillance and not research per se, ethical approval for the study protocol has been obtained from the Nepal Health Research Council.

**Timeline of field work:**

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<tr>
<th>SN</th>
<th>Activities</th>
<th>Start date</th>
<th>End date</th>
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<tbody>
<tr>
<td>1</td>
<td>Recruitment of field investigators</td>
<td>6-Sep-20</td>
<td>11-Sep-20</td>
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<tr>
<td>2</td>
<td>Training field investigators</td>
<td>11-Sep-20</td>
<td>14-Sep-20</td>
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<td>3</td>
<td>Pretest data collection tools (during training)</td>
<td>11-Sep-20</td>
<td>14-Sep-20</td>
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<td>4</td>
<td>Logistic preparation and departure for the field</td>
<td>15-Sep-20</td>
<td>17-Sep-20</td>
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<td>5</td>
<td>Pilot Field Work and review</td>
<td>17-Sep-20</td>
<td>20-Sep-20</td>
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<td>6</td>
<td>House Hold interview and blood sample collection</td>
<td>21-Sep-20</td>
<td>25-Sep-20</td>
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<td>7</td>
<td>Supervision by EDCD/WHO</td>
<td>17-Sep-20</td>
<td>25-Sep-20</td>
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<td>8</td>
<td>Concurrent Data coding /editing /cleaning</td>
<td>21-Sep-20</td>
<td>30-Sep-20</td>
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