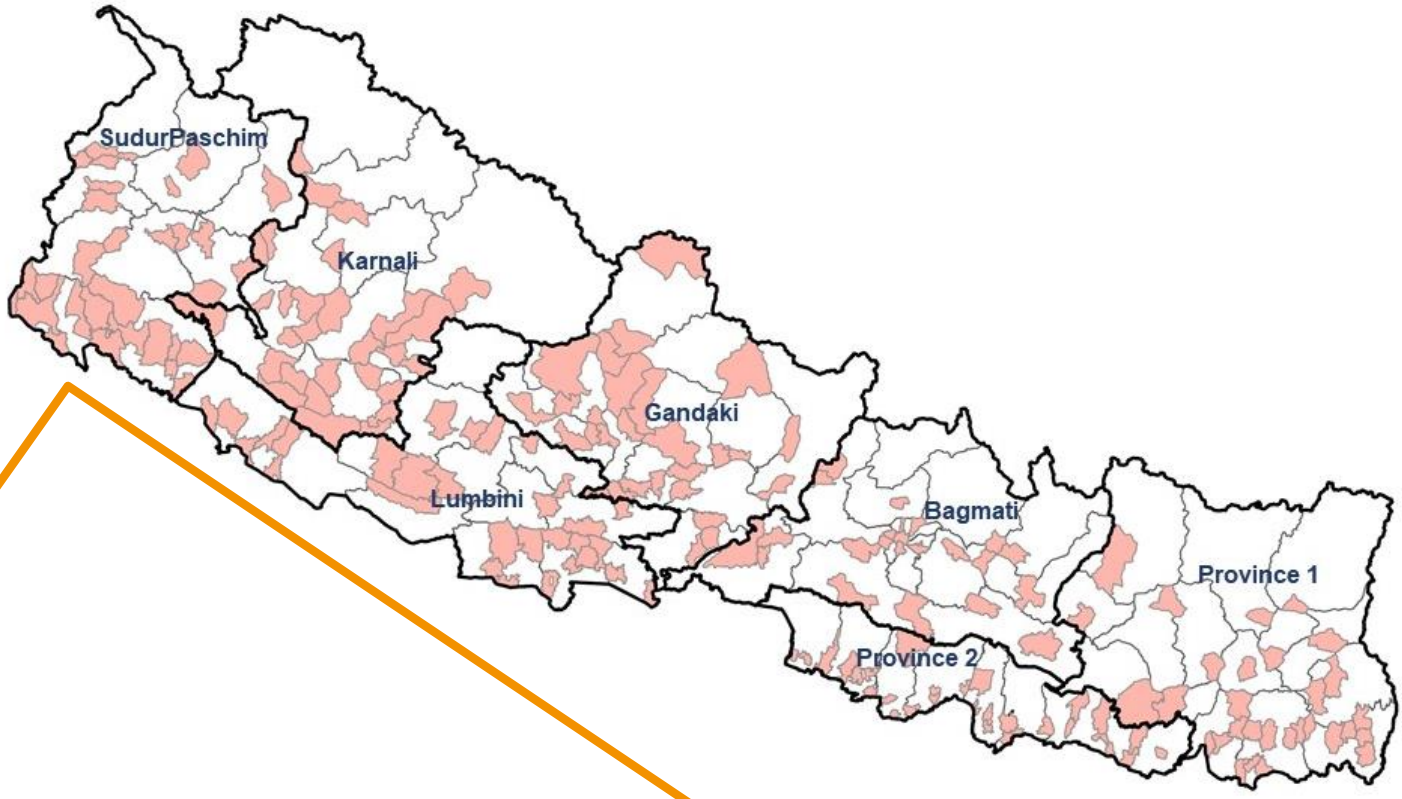


ENHANCED SURVEILLANCE ON SERO-PREVALENCE OF SARS-COV-2 IN GENERAL POPULATION

Nepal, 2020

Government of Nepal
Ministry Of Health and Population

Abbreviations

BSL	Bio Safety Level
ELISA	Enzyme Linked Immunosorbent Assay
HCW	Heal Care Worker
HIV	Human Immuno-Deficiency Virus
IPC	Infection Prevention and Control
NHRC	Nepal Health Research Council
NIBSC	National Institute for Biological Standards and Control, United Kingdom
NPHL	National Public Health Laboratory
NPR	Nepali Rupees
PHSM	Public Health and Social Measures
RT-PCR	Reverse Transcriptase – Polymerase Chain Reaction
SARS-CoV-2	Severe Acute Respiratory Syndrome Coronavirus 2
WHO	World Health Organization

CONTENTS

Acknowledgement

The national Sero-prevalence survey was jointly undertaken by the Epidemiology & Disease Control Division (EDCD); and the National Public Health Laboratory (NPHL) of the Department of Health Services (DoHS), Ministry of Health& Population (MoHP), Government of Nepal (GoN) in collaboration with the World Health Organization engaging all three levels of the organization – the country office for Nepal, the South East Asia Regional Office (SEARO) and the Head Quarters (HQ) at Geneva. The support and guidance of the senior leadership of these implementing entities is duly acknowledged.

The study protocol benefitted from the technical and ethical review and approval processes of the Nepal Health Research Council and the WHO-SEARO and HQ technical review processes for the WHO "UNITY" studies; and the inputs on and review of the sample weights used in the analysis by WHO-HQ experts.

The WANTAI SARS-CoV-2 Total Antibody detection ELISA kits used for testing the sera collected through the survey and the SARS-COV-2 serum panels from the National Institute for Biological Standards and Control (NIBSC), UK used for standardizing the tests were supplied at no cost by the WHO Unity Studies, a global sero-epidemiological standardization initiative, with funding to WHO by the COVID-19 Solidarity Response Fund and the German Federal Ministry of Health (BMG) COVID-19 Research and development Fund. The Operations Support & Logistics units of the WHO Health Emergencies Program and the procurement units at all three levels of the organization facilitated the shipment of the kits and serum panels.

The expenses incurred for the field component of the Sero-prevalence survey which included the contracting of two field research organizations - Center for Molecular Dynamics Nepal (CMDN) and Center for Health and Disease Studies (CHDS), Nepal for supporting the interviews, data collection and data management; laboratory consumables; training of the field investigators and phlebotomists; and epidemiology and laboratory expert consultants engaged in assisting the technical officers of the WHO Country Office and the officials of the EDCD and NPHL in planning and implementing the survey were supported through a grant from the Foreign, Commonwealth & Development Office (FCDO), Government of UK to the WHO Country Office for Nepal for COVID-19 response.

The engagement, facilitation and support of the Provincial Health Directors, District Health Officers, Health Coordinators of Municipalities; Ward chairs and the Female Community Health Volunteers was instrumental in the smooth implementation of the sero-survey at the municipal wards / clusters selected. Selected specially trained phlebotomists from the primary health centers and district hospitals were engaged

to collect blood from the study subjects and process the sera for transportation which was facilitated by the field teams of the WHO Country Office for Nepal.

The study findings were presented to, reviewed and endorsed by a national expert group convened by the Chair of the Epidemiology & Surveillance pillar of the MoHP, GoN Incident Command System for COVID-19 response. It also benefited from a special statistical review by the Director General – Central Bureau of Statistics, National Planning Commission, GoN.

The unstinting and generous support and cooperation extended by the households and individual members of the households selected as study subjects by willingly providing the information and blood samples needed which ensured the successful completion of this endeavor is duly acknowledged and highly appreciated.

EXECUTIVE SUMMARY

BACKGROUND

The national Sero-prevalence survey of Nepal for COVID-19 was conducted from 09 to 22 October 2020. The primary objective was to estimate the Sero-prevalence of SARS-CoV-2 in the general population of Nepal by measuring total antibodies which peak around three weeks after infection. The findings of this survey will help national authorities to initiate more targeted public health measures to control and prevent COVID-19.

METHODOLOGY

A multistage cluster sampling method was used to select a representative sample of the population. The administrative wards were considered as clusters, and 30 were selected from each province using probability proportionate to the size. In each cluster, 200-250 households were expected to be included. If the number of households in a cluster was high, the cluster was divided into segments applying a grid on a cluster map and one segment was selected randomly for the survey. Then fifteen households were randomly selected from each selected cluster (or the segment) for inclusion in the survey. One eligible member of the selected household who was residing in Nepal for at least four weeks prior to the survey date was enrolled by a random selection. The eligible age limit was defined as six months or older considering the operational feasibility of blood sample collection. The estimated sample size for this survey to obtain a precise estimate of national level Sero-prevalence was 3,150. The sample size calculation parameters were 1% expected Sero-prevalence, 1.5% margin of error, 5% alpha error with a design effect of 2. The sample size was inflated by 10% for non-response.

SAMPLE COLLECTION AND LABORATORY PROCEDURE

A group of trained, experienced phlebotomists selected from primary health centers were involved in drawing blood samples. A single phlebotomist collected blood samples from a given cluster. Samples were collected into biochemistry vacutainer tubes. Serum was separated and transported to the National Public Health Laboratory

as soon as possible under cold chain (2°-8°C) conditions. The samples were tested for COVID-19 total antibody presence using WHO-approved WANTAI SARS-CoV-2 antibody ELISA kits. This ELISA test detects both IgM and IgG antibodies for SARS-CoV-2 virus. The sensitivity and specificity of the test is 96.7% and 97.5% respectively. This test does not have any known cross-reactivity with antibodies to other coronaviruses and other types of the virus such as HIV, , dengue etc. A WHO-international laboratory consultant supervised all assays at the National Public Health Laboratory (NPHL).

RESULTS

The weighted estimate of national Sero-prevalence for COVID-19 in Nepal was 14.4% (95%CI 11.8-17.0). Relatively higher Sero-prevalence of 15.8% (95%CI 13.0-19.1) was observed in males compared to 12.2% (95%CI 9.0-16.4) females. The COVID-19 Sero-prevalence distribution among the three ecological regions of mountains, hills and terai were 4.6% (95%CI 2.2-9.5), 11.7% (95%CI 7.5-17.9) and 17.7% (95%CI 14.7-21.2), respectively. Among frontline healthcare workers/security personnel, Sero-prevalence was 10.8% (95%CI 5.8-19.1).

The risk analysis identified that the terai region (OR = 4.31) was found to have four times greater risk of exposure to infection than the mountain region. Similarly, the hill region (OR = 2.91) was found to have three times greater risk than the mountain region.

The survey data was analyzed using survey design weights and post-stratification with the provincial population.

CONCLUSIONS

The present study is the first serosurvey conducted from a planned series of Sero-prevalence studies for COVID-19 in Nepal. The estimated national Sero-prevalence was 14.4% (95% CI, 11.8-17.0). The actual infection prevalence is far greater than the observed case prevalence observed through routine surveillance systems. However, a substantial proportion of Nepal is still susceptible to COVID-19. Terai regions are comparatively more affected by COVID-19 than the hill regions or mountain regions. Therefore, greater protection of the hill and mountainous region populations may play a role in controlling and preventing COVID-19 in these areas.

RECOMMENDATIONS

Given the survey findings which suggest that case detection is low, there is a need for greater action to support testing expansion for the detection of asymptomatic and mildly symptomatic cases in the community, as well as rigorous and systematic contact tracing in an effort to break the chain of transmission. The survey findings suggest that a high proportion of the population is still susceptible (approximately 85%) to COVID-19. Therefore, there is a need for stricter adherence to public health and social measures to continuously mitigate SARS-CoV-2 transmission in the community.

Within the eco-zones, efforts must be made to protect mountainous regions from case surges through the minimization of travel to these areas while being mindful of the economic impacts to the tourism industry. The elderly population of older than 75 years had the lowest Sero-prevalence, yet greatest mortality in comparison to other age groups according to routine surveillance data. This suggests that the elderly population should be shielded and considered a priority group for vaccination. The survey suggests that females may have had lower testing than males. This suggests that more action is needed to support equitable testing amongst both females and males.

REPORT

BACKGROUND

Coronavirus disease 2019 (COVID-19) caused by Severe Acute Respiratory Syndrome Coronavirus-2 (SARS-CoV-2) emerged as a pandemic and spread to more than 200 countries, claiming to this date globally more than 90 million cases and nearly two million deaths[1]. The numbers are increasing daily as the novel virus spreads with a high attack rate and effective reproduction of more than one[2,3]. The proportion of asymptomatic cases amongst those who test positive ranges from 20 to 75 percent in the general population in different settings at different stages of transmission[4]. Given the possible high proportion of asymptomatic patients, estimating the true incidence of infection remains complex. When it comes to estimates of caseload in a community, facility-based surveillance for overtly symptomatic patients is likely to miss mild and asymptomatic cases, giving a lower caseload than the reality[5,6]. However, one can determine the extent of infection in the general population along with other valuable estimates like, age-specific point prevalence, proportion of asymptomatic or subclinical infections through targeted, antibody-based serologic testing via a statistically robust random sample survey, the precise reason many countries undertook sero-surveillance at various stages of transmission[7,8].

A cross-sectional sero-sampling survey provides a one-time snapshot of exposure/infection profile of the population. It estimates the exposure of SARS-CoV-2 in the general population by measuring antibodies in blood (IgG or total antibodies - IgM and IgG) which usually peak around 3-5 weeks after infection. This study was conducted with this complementary approach to understand the extent of COVID-19 infection in the community in Nepal. This is with the understanding that testing for active cases had initially targeted persons returning from India and other countries and was not always systematically within the general population based on pre-set criteria for testing.

Higher levels of IgM and IgG are found to be positive in the second and third week of illness as identified by ELISA[9,10]. IgM antibodies begin to decline and reaches lower levels by week 5 and almost disappears by week 7, whereas IgG persists beyond 7 weeks.

Sero-prevalence studies in the US found prevalence of antibodies in the general population ranging from 1.8% to 14%[11]. Results of a national Sero-prevalence survey of COVID-19 conducted between May and June 2020 in India estimated a 0.73 percent (95%CI, 0.34-1.13] national prevalence[12]. A subsequent Sero-prevalence survey conducted in Northern India identified a prevalence of 3.6% (95% CI 2.9% to 4.3%)[13].

Between 9th March 2020 to 10th April, the study conducted in Wuhan, China revealed Sero-prevalence between 3 to 4 percent[14]. In France at the end of February 2020, Sero-prevalence was initially 0.4%. However, approximately four weeks later at the end of March 2020, Sero-prevalence reached 4.1% at the exponential part of the epidemiological curve. At the end of April 2020, Sero-prevalence then reached 4.9%[15]. In an early study in Geneva, Switzerland, Sero-prevalence indicating infection was found to be more than ten times the prevalence detected through clinical syndromic surveillance[16].

JUSTIFICATION

As of 19 September 2020, COVID-19 had been reported from all 77 districts of Nepal, and 61,593 cases had been confirmed through Reverse Transcriptase-Polymerase Chain Reaction (RT-PCR) tests (0.1% – 0.2% prevalence) with a case fatality ratio of 0.63%[1]. The number of cases and deaths were increasing. Health leadership in Nepal was keen to have an estimate of SARS-CoV-2 exposure in the population in each province as well as the nation as a whole. High numbers of asymptomatic cases correlated with the presence or absence of antibodies with a history of COVID-19 like symptoms can help understand the extent of asymptomatic disease transmission in the community. Similarly, history of international travel in those having antibodies may indicate the role of importation of the infection into the country vis-à-vis indigenous transmission.

Nepal's targeted Sero-prevalence study was designed to help inform decisions about the role of certain predictor variables as possible determinants of transmission and inform decisions about the risk of indigenous transmission in the country. The study also sought to identify base line estimates so that similar studies could be repeated at periodic intervals to assess progression of transmission in the community and to monitor the effectiveness of intervention measures. The entirety of this information has the potential to guide the formulation of necessary actions as well as enhance preparedness capacity.

As blood samples were collected through a robust sampling technique, the study also took the opportunity of testing the residual serum samples after SARS-CoV-2 testing for measles and rubella Immunoglobulin-G (IgG). These results are not reported here.

GENERAL OBJECTIVE

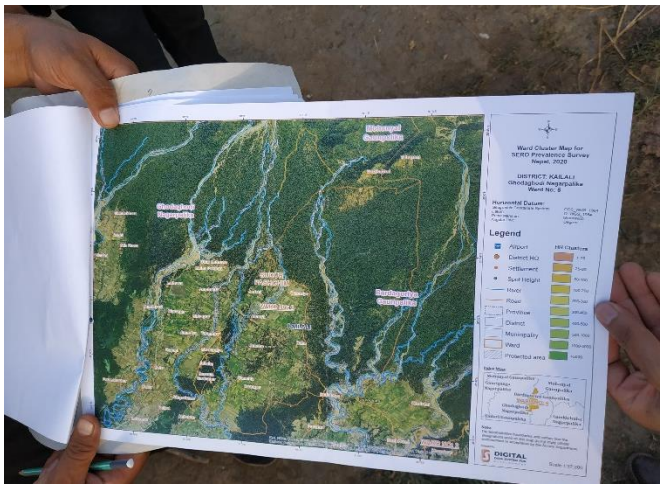
To determine Sero-prevalence of SARS-CoV-2 in the general population of Nepal.

Primary objective

- To determine the Sero-prevalence for SARS-CoV-2 infection in each province of Nepal by treating each of the seven provinces as a sample stratum.
- To describe age, sex and place, comorbidity, history of travelling and symptoms that are specific to Sero-prevalence, as determined by seropositivity.

Secondary objectives

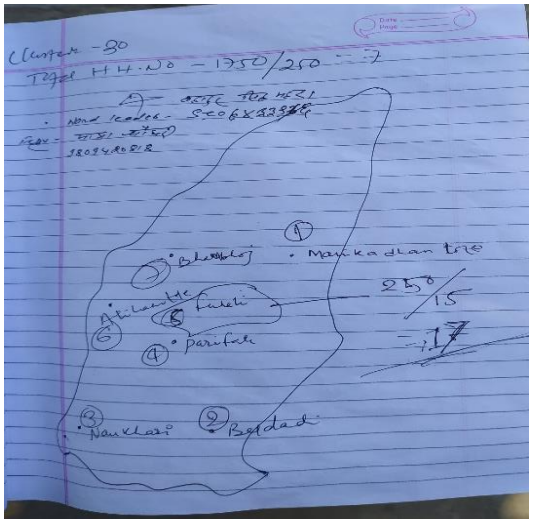
- To describe the epidemiology of COVID-19 in Nepal including asymptomatic transmission by correlating seropositivity with history of symptoms or signs of COVID-19, which may inform decisions upon future preparedness and response interventions.
- To determine Sero-prevalence for SARS-CoV-2 infection in the three ecological areas of Nepal (terai, mountains and hills) by pooling samples from all strata (7 provinces).
- To determine Sero-prevalence for other diseases of national public health importance (e.g. measles-rubella) from the aliquoted samples collected for SARS-CoV-2 testing.



Pic 1: Geospatial Maps of the selected cluster

METHODS

This was a population based, province stratified, nationwide cross-sectional study. The study was adjunct to the national surveillance program for COVID-19 and was designed with a cluster sampling method of a statistically robust random sample of the population, aged 6 months or above irrespective of past history of COVID-19 infection, symptoms and other medical conditions. The primary sampling unit (PSU) or cluster was a ward (newly formed) in a municipality, known as a *palika*.



Pic 2: Sketched map with the boundary

Sampling

The sampling method is aligned with WHO Immunization Cluster Survey methodology[17]. The administrative wards were considered survey clusters and thereby the primary sampling unit. In stage one, 30 clusters or wards were selected in each province with probability proportionate to size, the number of households in the clusters indicating the size. In stage two, clusters were divided into segments of approximately 200-250 households by applying a grid on the high-quality cluster/Ward GIS maps drawn to scale of 1:24,100. In stage three, 15 households were selected from each selected cluster or cluster segment using sequential random sampling method. Finally, within the household, a Modified Kish Grid table was used to select an individual out of all eligible members of a selected household for inclusion in the survey.

Study Population

The study population consisted of any person aged six (6) months or older and living in Nepal for a continuous period of at least 4 weeks prior to the first blood sampling date. The population under 6 months was excluded for operational difficulties in getting a blood sample and also because young infants have not been found to be significant source of COVID-19 infection. The population between 6 to 12 months was included as it was important to assess their seropositivity and immune status for COVID-19 as well as to measles and rubella.

The sampling unit was the one individual at or above 6 months randomly selected from every selected household. Persons were required to be permanent residents of Nepal. If the person was not a permanent resident, the person was required to have had a four week stay in Nepal prior to the blood sample collection date. This was irrespective of age or prior history of COVID-19 infection of the person, family members or other contacts. Suspected or confirmed prior COVID-19 infection was not considered as an exclusion criterion.

Exclusion criteria included refusal to give informed consent or contraindication to venepuncture. Additionally, to allow for enough time for exposure to infection and development of antibodies (IgM or IgG or other types), any person who had arrived in the country less than 4 weeks prior to the blood sample collection date was excluded.

Pic 3: Data Collection

Sample size

Four hundred and fifty persons from each province and 3150 from all 7 provinces in total were included in the sample. This sample size was estimated using the following parameters:

- Expected prevalence of seropositivity = 1%. This was estimated using other Sero-prevalence studies[16] and took into consideration the very low impact that COVID-19 had had in the Nepali health system at the time of planning, suggesting an overall low prevalence*
- Margin of error = 1.5%Confidence interval: 95%
- Design effect: 2
- Nonresponse rate: 10%

The sample size for one province was calculated at 400 and adding 10% nonresponse resulted in 450 for each province. From each province 30 clusters were selected with 15 people randomly selected in each cluster. The total persons selected from each province were 450 (30 X 15) with an overall national (7 provinces) sample size of 3150.

* This serosurvey was planned to be undertaken in June 2020. The expected seroprevalence used for the sample size calculation reflects the probable seroprevalence at that point in time. The survey was delayed due to administrative and logistical issues, which resulted in the central survey team unable to change the sample size estimation parameters and sample size. This is also due to the understanding that at this time ERC approval had already been received.

Data and sample collection from individuals selected

Trained enumerators undertook survey implementation for data collection, ensuring Infection Prevention and Control (IPC) measures during the survey. Informed consent was obtained. When the participant was a minor, assent was taken from the child's caregiver/legal guardian. Following informed consent, data collection was undertaken by enumerators using a structured questionnaire and a blood sample was obtained by a phlebotomist.

Pic 4: Sample Collection during the survey

The following process was adapted for collected blood samples. Serum was separated from whole blood through centrifugation after clot retraction and was stored and shipped at +2 to +8°C to NPHL within seven days of collection. The collection of blood samples followed specimen collection guidance in Nepal as followed for measles surveillance and other sero-surveillance investigations. A separate standard operational procedure had been developed for this. Processing of collected blood samples were undertaken with safe handling practices and spill decontamination procedures.

Serum samples were tested at the NPHL for the presence of COVID-19 virus specific total antibodies using WANTAI SARS-CoV-2 Ab ELISA (Beijing Wantai Biological Pharmacy Enterprise Co., Ltd, Beijing, China). in biosafety level -2 (BSL-2) laboratory. The sensitivity and specificity of the test is 96.7% and 97.5% respectively[18]. A National Institute for Biological Standards and Control (NIBSC), United Kingdom anti-SARS-CoV-2 sera panel obtained from WHO was also tested along with the samples as part of the laboratory quality control[19].

Pic 5: Labelling of the collected sample at the local health facility

Laboratory Testing and Data analysis

Survey design weights were calculated using selection probabilities of the multiple stages of sampling. The response rate in the seven provinces ranged from 96.7% to 100% with a non-response rate of 1.4%. Given the overall low nonresponse rate, weights were not adjusted for nonresponse. Survey weights were post stratified using provincial population values to estimate population totals. National level Sero-prevalence was adjusted for age structure distribution projected for the year 2020 by the Central Bureau of Statistics, Nepal[20]. Base R version 4.0.2[21], which included survey 4.0[22], epiR 1.0-15[23] and epitools 0.5-10.1[24], were used for data analysis.

Pic 6: Interview with the participants

The confidence level of 95% was used for interval estimates. A p-value of 0.05 or less was considered statistically significant.

Determination of possible risk factors was one of the items in the analysis plan. Variables subjected to risk factor analysis included age, sex, education, occupation, terrain, and household income.

All significant crude Odd Ratios were subjected to logistic regression analysis accounting for survey weights.

RESULTS

The total sample size was 3,150. Among these, 110 serum samples were not available for laboratory testing of these 110 eligible study participants, 13 had not consented for the survey and the from the remaining 97 (nonresponse rate <1%) samples were either haemolysed or the serum quantity was insufficient for ELISA testing. The 3040 available samples were subjected to laboratory testing. There were no major differences between responders and non-responders and those for whom samples were available for testing and those whose samples could not be tested. Table 1 summarizes the findings of the results obtained through this laboratory testing.

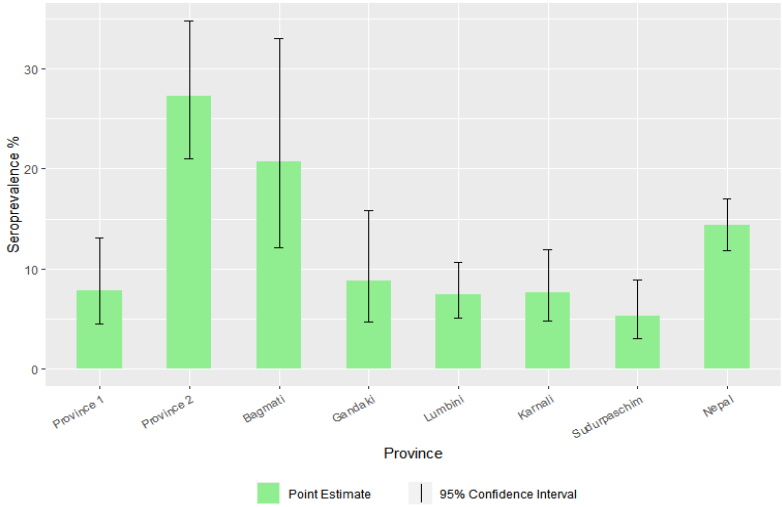


Figure 1: National and Provincial Seroprevalence of COVID-19, Nepal

Table 1: National and Provincial Sero-prevalences of COVID-19, Nepal

Province	Total Tested	Positive	Sample Percentage	Weighted Percentage	Lower 95%CI	Upper 95% CI
Province 1	442	44	10.0	7.8	4.5	13.1
Province 2	438	114	26.0	27.3	21.0	34.8
Bagmati	431	79	18.3	20.7	12.1	33.0
Gandaki	438	36	8.2	8.8	4.7	15.8
Province 5	419	32	7.6	7.4	5.1	10.7
Karnali	429	31	7.2	7.6	4.8	11.9
Sudurpaschim	443	26	5.9	5.3	3.0	8.9
Nepal	3040	362	11.9	14.4	11.8	17.0

$X^2 = 139.1$, df = 1, $p < 0.001$

According to Table 1, the national crude Sero-prevalence was 11.9%. The weighted percentage was 14.4% (95% CI, 11.8-17.0). Among the provinces, the highest Sero-prevalence was in Province 2 (27.3%) and the lowest Sero-prevalence was in Sudurpaschim (5.3%). Bagmati province had a Sero-prevalence of 20.7%.

Table 2: Sero-prevalence by Socio-demographic and selected risk factors, Nepal

Socio-Demography/Risk Factor	Total (%)	Weighted Sero-prevalence % (95% CI)	P-Value
Age			X^2 = 35.1, df = 9, **p = 0.01**
0-4		110 (3.6)	9.5 (4.6-18.6)
5-14		345 (11.3)	8.5 (5.4-13.3)
15-24		547 (18.0)	14.3 (10.1-19.8)
25-34		463 (15.3)	13.9 (10.5-18.1)
35-44		487 (16.0)	19.5 (14.4-25.9)
45-54		428 (14.1)	16.0 (10.9-22.9)
55-64		350 (11.5)	17.0 (12.0-23.6)
65-74		208 (6.8)	16.2 (10.3-24.7)
75-84		77 (2.5)	6.0 (1.6-20.5)
85+		25 (0.8)	2.1 (0.2-15.3)
Sex			X^2 = 7.8, df = 1, **p = 0.07**
Male		1744 (57.4)	15.8 (13.0-19.1)
Female		1296 (42.6)	12.2 (9.0-16.4)
Education level			X^2 = 8.7, df = 4, **p = 0.2**
None / informal		898 (29.5)	16.0 (12.5- 20.3)
Primary level		396 (13.0)	11.5 (8.1-16.2)
Secondary level		1,387 (45.6)	14.3 (11.2-18.1)
Secondary level plus		211 (6.9)	16.6 (11.0-24.4)
Data missing		148 (4.9)	9.5 (5.1-16.8)
Occupation			X^2 = 46.2, df = 6, **p < 0.001**
Agriculture		1,330 (43.8)	10.4 (8.2-13.1)
Service, Trade or Business		393 (12.9)	20.8 (15.1-28.0)
Home maker and Unemployed		445 (14.6)	20.3 (13.6- 29.2)
Students		457 (15.0)	14.0 (10.1-19.2)
Social engagement		92 (3.0)	19.2 (10.7-31.9)
Frontline workers: HCW & Security		122 (4.0)	10.8 (5.8-19.1)
Others & Data missing		201 (6.6)	12.4 (7.5-19.7)

Socio-Demography/Risk Factor	Total (%)	Weighted Sero-prevalence % (95% CI)	P-Value
Presence of co-morbidities			X^2 = 2.3, df = 1, p = **0.1**#
Yes		414 (13.6)	17.1 (11.5 – 24.7)
No		2626 (86.4)	13.9 (11.4 -17.0)
Geo-Eco region			X^2=36.1, df = 2, **p = 0.009**
Terai		1,312 (43.2)	17.7 (14.7-21.2)
Hill		1,477 (48.6)	11.7 (7.5- 17.9)
Mountain		251 (8.3)	4.6 (2.2-9.5)
Income level (NPR)			X^2 = 76.3, df = 4, **p < 0.001**
0-10,000		798 (26.3)	8.9 (6.7-11.7)
10,000-60,000		2121 (69.8)	14.8 (12.4-17.6)
60,000-120,000		91 (3.0)	31.1 (13.9-55.7)
120,000-240,000		17 (0.6)	53.7 (15.9-87.7)
240,000+		13 (0.4)	28.5 (6.4-69.8)
History of symptoms			X^2 = 2.9, df = 1, **p = 0.09**#
Yes		451 (14.8)	16.1 (11.3-22.5)
No		2589 (85.2)	14.1 (11.4-17.3)
Fraction of asymptomatic among those that tested positive*	297 (82.0)		85.8 (79.8-90.3)

* Among those who were never symptomatic # Unweighted

Almost half of the sample (48.0%) has a secondary level of education. Agriculture (46.8%) was the single largest group by occupation. Only 4.3% of the sample were frontline workers including healthcare workers and security personnel. Approximately 70% of the sampled households earned between 10,000 and 60,000 NPR per month. Approximately 14% of the sample reported having a diagnosed co-morbidity. Only 10 people reported having travelled abroad and only 9 people reported having been in contact with a confirmed Covid-19 case.

When examining age structure, it was identified that the economically productive age group experienced greater seropositivity compared to children and the elderly. Among the age groups in the economically productive category, 15-24 years and 35-

44 years had the lowest (14.3%) and highest (19.5%) Sero-prevalence, respectively. Among different education levels, the Sero-prevalence ranged from 11.5% to 16.6%. Sero-prevalence varied among occupations from 10.4% to 20.8%. It is noteworthy to mention that in our sample, Sero-prevalence among frontline workers was not very high (10.8%). Among geo-eco regions, mountainous populations had the lowest seropositivity with Terai communities having the highest. Approximately 15% of the survey participants had had symptoms of COVID-19. The Sero-prevalence among this group was 16.1%. Similarly, 85.2% of the sample never have symptoms suggestive of COVID-19 and had seropositivity of 14.1%. Among those that tested positive, 85.8% (95% CI, 79.8-90.3) were ever asymptomatic for COVID-19.

Table 3: Univariate risk factor analysis for IgM and/or IgG positivity, crude and weighted

Risk factor	Total Tested	Positive	Crude OR (95%CI)	p-value	Weighted OR (95%CI)	p-value
Age						
Children	455	35	0.55 (0.38-0.79)	**<0.01**	0.51 (0.34-0.76)	**0.001**
Elderly	310	28	0.66 (0.44-0.99)	**0.04**	0.72 (0.45-1.17)	**0.19**
Working	2275	299	**Reference**		**Reference**	
Sex						
Male	1744	232	1.38 (1.10-1.73)	**0.01**	1.35 (0.98-1.87)	**0.07**
Female	1166	130	**Reference**		**Reference**	
Eco Zones						
Terai	1312	222	4.06 (2.23-7.37)	**<0.001**	4.43 (2.09-9.38)	**<0.001**
Hills	1477	128	1.89 (1.03-3.47)	**0.04**	2.73 (1.15-6.48)	**0.02**
Mountains	251	12	**Reference**		**Reference**	
Education level						
Primary level	396	44	0.89 (0.61, 1.28)	**0.52**	0.88 (0.62-1.23)	**0.45**
Secondary level	1387	170	0.99 (0.77, 1.28)	**0.94**	1.05 (0.62-1.77)	**0.87**
Secondary level plus	211	25	0.95 (0.60, 1.51)	**0.84**	0.55 (0.29-1.03)	**0.07**
None / informal	898	111	**Reference**		**Reference**	

Risk factor	Total Tested	Positive	Crude OR (95%CI)	p-value	Weighted OR (95%CI)	p-value
Occupation						
Agriculture	1330	121	0.84 (0.46, 1.54)	**0.57**	0.96 (0.49-1.88)	**0.90**
Service, Trade or Business	393	74	1.95 (1.04, 3.65)	**0.04**	2.17 (1.00-4.68)	**0.05**
Home maker and Unemployed	445	69	0.84 (0.46, 1.54)	**0.18**	2.11 (0.90-4.94)	**0.09**
Students	457	52	1.08 (0.57, 2.05)	**0.82**	1.35 (0.58-3.11)	**0.48**
Social engagement	92	13	1.38 (0.61, 3.14)	**0.44**	1.96 (0.75-5.10)	**0.17**
Frontline workers: HCW & Security	122	13	**Reference**		**Reference**	
Income (NPR/month)						
>10,000	2242	1945	1.72 (1.30-2.28)	**<0.001**	1.97 (1.36-2.84)	**<0.001**
0-10,000	798	733	**Reference**		**Reference**	

Being of working group age has shown have a higher risk for acquiring infection at approximately 2 times the risk of the children's age group (OR 0.55 [95%CI, 0.38-0.79]), which is statistically significant.

When comparing eco-regions, persons residing in terai (OR 4.43 [95% CI, 2.09-9.38]) and hill (OR 2.73 [95% CI, 1.15-6.48]) regions had approximately four times and three times the risk, respectively, of acquiring the infection, which was statistically significant.

In comparison to frontline workers (healthcare workers and security personnel), service, trade and business occupations had twice (OR 2.17 [95% CI, 1.00-4.68]) the risk of acquiring the infection, which was statistically significant.

Incomes greater than 10,000 NPR per month showed a statistical significance for acquiring SARS-CoV-2 infection. Age and gender did not seem to be associated with Sero-prevalence (Table 3).

Table 4: Logistic regression for sero-positivity, weighted

Risk factors	OR (95%CI)	P Value
Age		
Children	0.51 (0.34-0.78)	**0.002**
Elderly	0.76 (0.47-1.23)	**0.27**
Working	**Reference**	
Sex		
Male	1.30 (0.94-1.79)	**0.11**
Female	**Reference**	
Eco Zones		
Terai	4.46 (2.09-9.53)	**<0.001**
Hills	2.79 (1.17-6.66)	**0.02**
	Reference	
Income (NPR/month)		
0-10,000	**Reference**	
>10,000	1.97 (1.37-2.83)	**<0.001**

The risk factors included in the logistic regression model were age, sex, ecozones, and income. After adjusting for these variables, the risk factors of the terai and hills eco zones, and children's age group remain statistically significant.

DISCUSSION

The survey extended from the second to third week of October 2020. It was considered that 14 days are needed for the COVID-19 seroconversion in humans to occur. Hence, Sero-prevalence observed in the survey represented the cumulative case load in the country up to 14 days prior to the mid-day (i.e. 16 October 2020) of the survey. The reflective cumulative caseload on October 3, 2020 was 82,450. These were the total cases which tested positive with RT-PCR and were reported from 68 quality-controlled laboratories around the country. The RT-PCR was offered freely for all those who were opt for testing. The observed population prevalence of COVID-19 based on routine surveillance data was 0.3%[1]. This is far less than compared to the survey Sero-prevalence estimate which was 14.4%. This indicates that the asymptomatic fraction of the COVID-19 caseload was higher. In fact, the estimated asymptomatic cases in this survey were 87.6% (95% CI, 84.6-90.1). This finding was supported by another independent survey conducted by National Health Research Council (NHRC) among home isolated patients where the asymptomatic fraction was 66% (*Personal Communication*). The under reporting amounted approximately to 50 times what was observed from routine surveillance data (0.3% caseload prevalence vs. 14.4% Sero-prevalence).

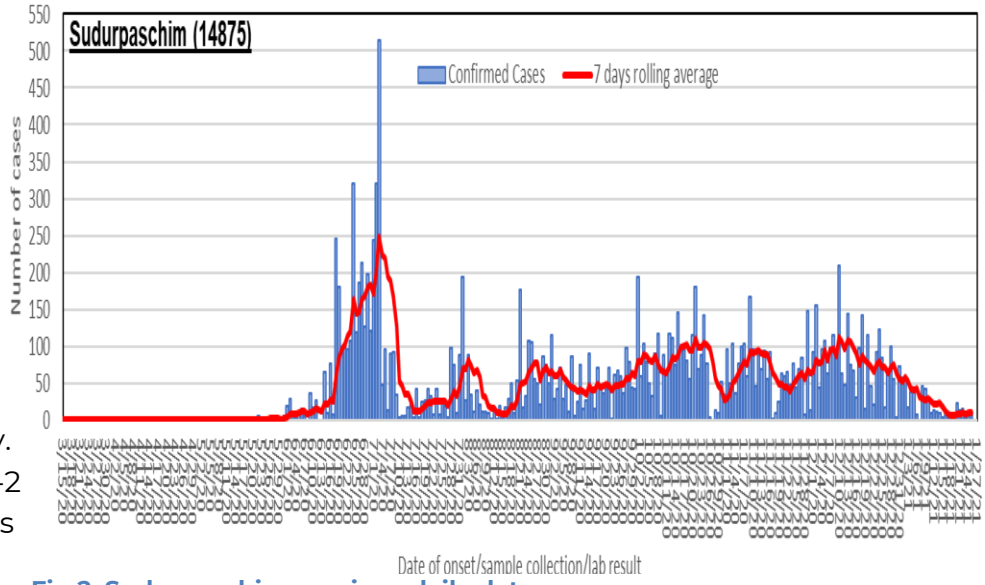


Fig 2: Sudurpaschim province daily data

We used an ELISA measuring total antibodies considering that it had the highest sensitivity in detecting anti-SARS-CoV-2 antibody. Hence, anti- SARS-CoV-2 total antibody ELISA is preferred over anti SARS-CoV-2 IgG ELISA for enhanced sero-surveillance of SARS-CoV-2 infection in population[8,25].

This survey was conducted at the peak of the general wave of the COVID-19 epidemic in the country which resulted in a continued severe burden on the health system as well as affected the socio-economic situation of the country. The survey findings suggest around 85% of the Nepali population are still susceptible to COVID-19, posing further threat to the economy if disease transmission is not managed in the community.

The Sero-prevalence of provinces vary from 5.3% in Sudurpaschim to 27.3% in Province 2. Prior to the survey, the highest Sero-prevalence was expected in Bagmati province from where more than 35% of the national caseload were reported around 60% of the daily caseload[26]. However, it was found that Province 2 had the highest Sero-prevalence (27.3%). This may indicate a surveillance and/or a testing gap in Province 2 which needs to be studied and addressed by provincial and federal government.

Another interesting correlation that can be observed in the serosurvey data are the low values of Sero-prevalence reported in the easternmost and westernmost provinces of the country. These areas are known for their relatively difficult geographic accessibility. In fact, the epi-curve of Sudurpaschim province indicates a repeated point source type exposure throughout year 2020 (Fig :2) indicating repeated reintroduction of the disease. This is probably due to travellers from outside of the country by border crossing. This should be taken into consideration for disease control activities by Sudurpaschim provincial health authorities.

Nepal's terrain can be grouped into three ecological areas according to height from sea level in ascending order including terai plains, hills and mountains. Among these areas, population Sero-prevalences varied and were strongly statistically significant. An interesting point identified that the Sero-prevalences were reciprocally varied to height from sea level. This observation may primarily occur due to accessibility difficulties and closed major tourist locations. This may include the area surrounding the Himalayan mountain range as a part of the country's strategy in response to COVID-19 prevention and control activities. It is advisable to carry forward this strategy in a public health perspective but questionable when considering its economic impact.

Surveillance data show that males (65%) were more affected than females (35%). However, the survey data showed a much closer seropositivity proportion of 15.8% in males in comparison to 12.2% in females, which was not statistically significant (P=0.07). This may indicate a gap in testing penetration to the female population which requires serious attention by federal and provincial health authorities as well as policymakers. This might indicate equity challenges in access to COVID-19 testing by females.

Routine surveillance data suggest that the most affected age groups were among the economically productive i.e., 15-74[26], which is reconfirmed by the serosurvey result. This may be a concern for political leadership and economists. The health sector may also be concerned because their mobility for work and recreation as well as poor adherence to public health and social measures have a greater impact on disease transmission in the community especially to the elderly population, a less affected age group[26].

This serosurvey also aimed to find out the risk factors for acquiring SARS-Cov-2 infection. A logistic regression model was fitted with selected risk factors. According to the result, residential eco-zones were found to be a major risk factor for acquiring the infection. It is evident that residents of terai region and hill region have approximately 4 times and 3 times the risk, respectively, when compared to mountain regions. This may be due to the population distribution pattern in Nepal as well as internal and external migration and geographic accessibility constraints which may also have played a part.

It is interesting to observe that higher income groups (>10,000 NPR) have approximately two times the risk than the income group which earns less than 10,000 NPR per month. This contradicts the findings of studies undertaken primarily in the United States. However, we believe this is one of the findings that requires further research to understand in Nepal and the south-east Asia regional context, where income may be closely linked to mobility and the initial wave of infection may have been due to the in-migrant population[27]. Sex did not appear to be a risk factor, reiterating that the ratio difference observed in the surveillance data is biased and probably indicates an inequity of access to testing.

Frontline workers (healthcare workers and security personnel) experienced less infection than other groups. This may be due to effective use of public health and social measures. One may need to be cautious given the fact that absence of data on fatalities among frontline workers, this might be a biased observation. In terms of school age populations of less than 15 years of age, this group had close to 10% positivity. This indicates the level of infection is higher than observed in the surveillance data[1]. Lower detection amongst school age populations may be due to greater levels of asymptomatic or mild disease.

Clearly COVID-19 can demonstrate different patterns of transmission phases of the epidemic curve in different zones of the same country depending on population and disease transmission dynamics. This study has demonstrated widely divergent infection prevalence where two provinces out of seven had a much higher prevalence of infection, as well as the Terai zone bordering India which also showed a higher prevalence. This should lead to targeted increased surveillance sensitivity including rapid laboratory turnaround in provinces or ecological zones where a higher transmission is expected.

The wider gap seen in province-2 (completely in Terai) between clinical case-based surveillance and Sero-prevalence should inform country program decisions to undertake thorough reviews and additional technical assistance to surveillance and lab systems to such areas.

Although vaccine intervention will take off soon in Nepal, nearly 85% of Nepalese population have had no exposure to SARS-CoV-2 and therefore remain vulnerable to a 'second wave'. Non-pharmaceutical interventions like physical distancing, wearing masks and hand hygiene must be practiced by all to prevent a resurgence of infection.

Conclusions

The Ministry of Health and Population has planned the Sero-prevalence study to understand the current seroconverted population proportion and adjust the public health and social measures employed for the COVID-19 control and prevention activities. The estimated national Sero-prevalence was 14.4% (95% CI, 11.8-17.0).

The present study is the first serosurvey conducted from a planned series of Sero-prevalence studies for COVID-19 in Nepal. The estimated national Sero-prevalence was 14.4% (95% CI, 11.8-17.0). The actual disease prevalence is far greater than the observed prevalence through routine surveillance systems. However, a substantial proportion of Nepal is still susceptible to COVID-19. Terai regions are comparatively affected more so by COVID-19 than the hill regions or mountain regions. Therefore, population movement restriction to hill and mountains may play a role in controlling and preventing COVID-19 in these areas.

Limitations:

One aspect of potential consideration is the difference between the proportions in sampled population and the actual population in terms of age and gender. This may be a result of selection bias due to deviation from the study protocol. Specifically, the sample included a greater proportion of males than females, as well as an older population in comparison to the national distribution. An evaluation of the survey involving interview of data collectors should be conducted in order to determine the cause of such deviation from the study protocol and whether this can be avoided in future surveys.

Recommendations

The survey findings suggest case detection is very low despite the higher proportion of symptomatic to total infected persons. This suggests the need for greater action from government to support the expansion of testing to detect asymptomatic and mildly symptomatic cases in the community and rigorous and systematic contact tracing to break the chain of transmission. Given the high proportion of susceptibility in the population (approximately 85%), this suggests the need for adherence to public health and social measures continuously to mitigate SARS-CoV-2 transmission in the community. To adjust proper public health and social measures, it is recommended

to adapt province and district specific transmission classification and situational assessments.

Province 2 and Bagmati have the highest Sero-prevalence, suggestive of community transmission. Therefore, stringent public health and social measures and active surveillance for cases, testing, and isolation and tracing of contacts with testing followed by isolation or quarantine is suggested in these provinces.

Among the eco-zones, it is recommended that efforts be made to protect mountainous regions from case surges by minimizing travel with simultaneous mitigation of economic impacts to tourism. This can be undertaken by application and implementation of more stringent PHSM and Points of Entry related interventions in Terai regions as well as through cluster containments at hotspots in hill regions.

According to sero-surveillance data, the elderly population of >75 years of age had lower Sero-prevalence. Despite a lower Sero-prevalence, the elderly was found to have higher mortality as identified from the surveillance data. This suggests that the elderly population should be considered a priority group for vaccination, thereby shielding this vulnerable group to prevent mortality and overburdening of health systems. The survey suggests that females may have had lower testing than males. This suggests that more action is needed to support equitable testing amongst both females and males.

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ANNEXURE 01: QUESTIONNAIRE USED DURING THIS SURVEY

COVID-19+ Serological surveillance Questionnaire Sep 2020							
Unique ID: *Standard reference list will be made available to enumerators.*	GPS Location	Prov	District	Palika	Ward	Cluster	Household
		Numeric Code [1-7]	Alpha-3 code [from IPD]	Numeric code [HIMS]	Numeric code [HMIS]	Numeric code (1-30)	Numeric code (1-15)
		#	X X X	# #	# #	# #	# #
Record names →							

BEFORE DATA COLLECTION , OBTAIN PERMISSION FROM THE HOUSEHOLD TO ASK QUESTIONS ABOUT MEMBERS IN THE HOUSEHOLD. MAKE SURE THAT THE PROVIDER KNOWS THAT YOU ARE NOT THERE TO EVALUATE HIM OR HER, AND THAT YOU ARE NOT AN "EXPERT" TO BE CONSULTED DURING THE SESSION.

If household agrees to provide information about household members, proceed to list members of household. If not STOP and record reason for refusal.

If the household refused to participate,

Reason for not participating (write reasons in points)	Other remarks

Date of interview			
	DAY	MONTH	YEAR
Reference date for exclusion (if staying in Nepal after this date)	DAY	MONTH	YEAR

	List of members in the household for selection of the subject to be recruited							
	Sl. Number of house (-1-15)		Area		Name of head x of household			
Sl No.	Name	Sex	Age in completed years	Age in months	Stayed in Nepal since before __/___/____ Do NOT include persons answering NO in Kish grid count	Rank order Number (youngest to oldest) of those who responded yes to column (6) and those who are 6 months or older age in col (5)	Relationship with the head of household	Person selected as per Kish grid (Only One) -
(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)
1					Yes/No			
2					Yes/No			
3					Yes/No			
4					Yes/No			
5					Yes/No			
6					Yes/No			
7					Yes/No			
8					Yes/No			
9					Yes/No			
10					Yes/No			
11					Yes/No			
12					Yes/No			
					YES =	Count=		

READ TO PROVIDER: Hello. I am …… I am representing Epidemiology and Disease Control Division, Ministry of Health and Population.

[PLEASE COPY PASTE CONSENT FORM INCLUDING TESTING FOR COVID-19 AND OTHER DISEASES OF OTHER PUBLIC HEALTH IMPORTANCE.]

CONSENT DETAILS TO BE TOLD

……

▫INSERT TEXT FOR CONSENT WITH SPACE FOR SIGNATURE

[SHOULD BE PART OF FORM]

IF MINOR, SELECTED, RECORD NAME ABD RELATIONSHIP OF GUARDIAN WITH MINOR

	DAY	MONTH	YEAR
INTERVIEWERS SIGNATURE			
RECORD WHETHER PERMISSION WAS RECEIVED	YES		
	NO		

If individual agrees to provide information proceed to collect information and blood sample. If not STOP and record reason for refusal.

Reason for not participating (write reasons in points)	Other remarks

INDIVIDUAL HEALTH QUESTIONNAIRE

Prov -- District– Palika -Ward – Cluster – Household – individual no.

 # XXX - ## ## ## ## ##

SPACE FOR BAR CODE

Name of responder if different from selected individual, (if same write same) – If different also write relation to selected individual	

Identifier information of the subject			
First Name		Surname	
Sex	▫ Male ▫ Female ▫ other		
Date of Birth	DD/MM/YY (English)		
Date of Birth	DD/MM/YY (Nepali)		
Age in Years			
Age in Months			
Telephone Mobile Number (1) (head of household)			
Telephone Mobile Number (2) of selected individual			
Country of Residence (Ask for countries where she/he is usually living)	▫ Foreign National Living in Nepal (.....) ▫ Non Resident Nepali (.....)		
Nationality			
Caste/Ethnicity group (HMIS)	▫Dalit ▫ Janjati ▫ Madeshi ▫ Muslim ▫ Brahman/Chhetri ▫ Others		
Occupation	▫ Health Care Worker including support staff ▫ Security Forces -Army, Police ▫ Agriculture ▫ Business or service with direct close interaction with clients (Hotel staff, school staff, beauty parlour staff, entertainment industry, priests, crematorium/burial site staff, commercial sex workers) NOTE EXACT NATURE OF WORK _____ ▫ Business or service with NO direct close interaction with clients – NOTE EXACT NATURE OF WORK _____ ▫ Study/Student ▫ House hold work/home maker ▫ Social work /community leaders/local self government ▫ Not Working / staying at home / ▫ Any other /specify		

Usual country of work	▫ Nepal ▫ India ▫ Others; If others, specify: _____
Travel History	Have you stayed (Night stay) in other district than you currently live. ▫ Yes ▫ No
Current COVID-19 Status	
Have you had close contact with anyone with confirmed COVID-19 infection ? **For all that is applicable –**	▫ Yes ▫ No ▫ Unknown If Yes, dates of last contact (DD/MM/YYYY): ___/___/___ (English) ___/___/___ (Nepali)
Since (15 January 2020/ 01 Magh 2076) or Magh Sankranti have you ever been tested for COVID-19?	▫ Yes ▫ No If Yes, dates of last testing (DD/MM/YYYY): ___/___/___ (English) ___/___/___ (Nepali)

COVID-19 test record

SL #	Type of test	Date of test	Date result	Lab name / Hospital name	Test result
1.					
2.					
3.					
4.					
5.					
6.					
7.					

Symptoms History	
Did you have any of the following symptoms at any time, since 15 th January 2020/ 1st Magh 2076? Fever, Dry Cough, Difficulty in breathing, Runny nose, General Weakness, Conjunctivitis (red eye), Tiredness, Headache, Muscle ache/ pain, Sore throat, Nausea, Vomiting, Diarrhoea, New Loss of Smell (Anosmia), New Loss of Taste (Ageusia) **(Tick mark all the symptoms, reported)**	
Did any of these symptoms require you to seek attention from a health worker/facility?	▫ Yes ▫ No
Did any of these symptoms require you to absent from work/school?	▫ Yes ▫ No
Did any of these symptoms made you isolated at home or a facility	▫ Yes ▫ No
Did any of these symptoms require you to be hospitalized?	▫ Yes ▫ No
During hospitalization, did you have supplemental oxygen?	▫ Yes ▫ No
Were you shifted to an Intensive Care Unit (ICU)?	▫ Yes ▫ No
Were you put on ventilator?	▫ Yes ▫ No

CODING FOR PROBABLE/CONFIRMED COVID-19 – TO BE DONE BY DESIGNATED MEDICAL PROFESSIONAL AFTER FIELD DATA COLLECTION

BASED ON ABOVE DID THE PERSON HAVE SYMPTOMATIC COVID-19 BETWEEN 16 JAN 2020 AND DATE OF SURVEY: YES/NO
IF YES, WHAT WAS THE MOST PROBABLE START DATE OF SYMPTOMS/SIGN: ____/ ___ / _____ END DATE OF SYMPTOMS/SIGN: ____/ ___ / _____
FINAL CODING FOR COVID-19 STATUS: CONFIRMED / PROBABLE /SUSPECTED / NOT COVID-19 [WHO CASE DEFINITION TO BE FOLLOWED]

TRAVEL HISTORY

From Magh Sankranti (16 Jan 2020) to till date, did you undertake any travel into or out of the country? If in and out of same country twice, record as two countries below.	▫ Yes ▫ No
If yes, then duration by date	From ..... To.....
Country/ies name 1:	Date in ----------

	Date out----------
Country/ies (1) name 2:	Date in ---------- Date out----------
Country/ies (1) name 3:	Date in ---------- Date out----------
Country/ies name 4:	Date in ---------- Date out----------

CO-MORBIDITIES	
Do you have a diagnosis of **(Tick mark all the diagnosis, reported)**	
Obesity	▫ Yes ▫ No
Diabetes	▫ Yes ▫ No
Hypertension	▫ Yes ▫ No
Cardiovascular disease,	▫ Yes ▫ No
Respiratory disease	▫ Yes ▫ No
Cerebrovascular disease	▫ Yes ▫ No
Malignancy (cancer)	▫ Yes ▫ No
Chronic kidney disease	▫ Yes ▫ No
Liver disease	▫ Yes ▫ No

Fever-Rash and Immunization HISTORY

1. Have you suffered from fever and rash now or in past 4 weeks? Y/N, (Dadura/Khasra/Gosain/Devi/Mata/):

2. If Yes, since when did the rash start –
   DD/MM/YY (NEP) ____ / _____ / _____
   DD/MM/YY (ENG) ____ / _____ / _____

3. Was a blood sample taken for fever and rash test for measles during this recent episode? – Y/N

4. If yes, when was it taken?
   DD/MM/YY (NEP) ____ / _____ / _____
   DD/MM/YY (ENG) ____ / _____ / _____

5. IMMUNIZATION HISTORY

Type of vaccine	Immunized? Y/N/Unknown	If YES, dose no.	Dose date	Source of Info (Card/ recall/ register)
Measles / Measles-rubella	Y/N/Unknown	1.		
Measles / Measles-rubella	Y/N/Unknown	2		
Measles / Measles-rubella SIA	Y/N/Unknown	1/2/3/...	Last dose date:	
BCG	Y/N/Unknown	1.		
DTP/Pentavalent	Y/N/Unknown	1.		
DTP/Pentavalent	Y/N/Unknown	2.		
DTP/Pentavalent	Y/N/Unknown	3.		
OPV	Y/N/Unknown	1.		
OPV	Y/N/Unknown	2.		
OPV	Y/N/Unknown	3.		
OPV in SIA	Y/N/Unknown	1/2/3/...	Last dose date:	
IPV	Y/N/Unknown	1		
IPV	Y/N/Unknown	2		
JE vaccine	Y/N/Unknown	1		
JE in SIA	Y/N/Unknown	1/2/3/...	Last dose date:	

LABORATORY RESULTS	
Person Unique Id (Copy entre	
Laboratory Identification No.	

Sample Collection	
Date of Sample collection	DD/MM/YY
Time of Sample Collection	
Type of Sample	▫ Blood ▫ Dried Blood Spot (DBS)
Name of Phlebotomist	
Centrifugation done at	..... Date ..... Time.....
Date sample dispatched to NPHL	
If not dispatched same day where the sample was stored	Place, duration, temp.

Laboratory (COVID total antibody test)	
Date of Sample received at NPHL	DD/MM/YY
Quality of the sample	▫ Good ▫ Hemolyzed ▫ Insufficient volume ▫ No label
Type of Serological Assay	
Serology Result for COVID-19	▫ Positive ▫ Negative ▫ Indeterminate or equivocal
OD value (corrected, if applicable)	
Date of test	DD/MM/YY

Laboratory (Measles IgG)		
Date of Sample received at NPHL	DD/MM/YY	
Quality of the sample	▫ Good ▫ Hemolyzed ▫ Insufficient volume ▫ No label	
Type of Serological Assay		
Serology result for measles	▫ Positive ▫ Negative ▫ Indeterminate or equivocal	
OD value (corrected, if applicable)		
If equivocal, result of repeat test	▫ Positive ▫ Negative ▫ Indeterminate or equivocal	Positive ▫ Negative ▫ Indeterminate or equivocal
OD value (corrected, if applicable)		
Date of test	DD/MM/YY	
Laboratory (Rubella IgG)		
Date of Sample received at NPHL	DD/MM/YY	
Quality of the sample	▫ Good ▫ Hemolyzed ▫ Insufficient volume ▫ No label	
Type of Serological Assay		
Serology result for rubella	▫ Positive ▫ Negative ▫ Indeterminate or equivocal	

OD value (corrected, if applicable)		
If equivocal, result of repeat test	▫ Positive ▫ Negative ▫ Indeterminate or equivocal	Positive ▫ Negative ▫ Indeterminate or equivocal
OD value (corrected, if applicable)		
Date of test	DD/MM/YY	

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