PEER REVIEW HISTORY

ARTICLE DETAILS

TITLE (PROVISIONAL)	Vaccine Preventable Disease Seroprevalence In a Nationwide Assessment of Timor-Leste (VASINA-TL) - study protocol for a population-representative cross-sectional serosurvey
AUTHORS	Arkell, Paul; Sheridan, Sarah L; Martins, Nelson; Tanesi, Maria; Gomes, Nelia; Amaral, Salvador; Oakley, Tessa; Solano, Vanessa; David, Michael; Draper, Anthony; Sarmento, Nevio; da Silva, Endang; Alves, Lucsendar; Freitas, Carlito; Machado, Filipe; Gusmão, Celia; da Costa Barreto, Ismael; Fancourt, Nicholas; Macartney, Kristine; Yan, Jennifer; Francis, Joshua

VERSION 1 – REVIEW

REVIEWER	Wang, Wei Food and Drug Administration, Division of Biostatistics, Center for Devices and Radiological Health
REVIEW RETURNED	15-Mar-2023

GENERAL COMMENTS	In this manuscript, the authors presented study protocol for a population-representative cross-sectional serosurvey to evaluate the Vaccine Preventable Disease Seroprevalence in a Nationwide Assessment of Timor-Leste (VASINA-TL). I mainly focused on the review of the statistical analysis and sample size calculation sections for this protocol based on my expertise. The data analysis presented in this protocol is generally acceptable and I have several comments about the sample size section presented on Page 8 to page 10, specifically, 1) The sample size calculation formula presented in the first line on Page 10 is not correct, the Z value was not included in the formula, please check and correct it. 2) Although the authors listed the non-response rate as 15% in Table 2, I do not believe the non-response rate was considered and used in the sample size calculation, please double check and confirm that. 3) It is not clear why the coefficient of variation was listed in Table 2 as one parameter of the sample size calculation. Please provide explanation about it. 4) It is recommended that the authors presented the sample size calculation formula in a standardized format which is easy to read and understand for the readers, for example, the authors may use the “inserted equation” function provided by Microsoft word to present the sample size calculation formula in the manuscript.

REVIEWER	Zirimenya , Ludoviko MRC/UVRI and LSHTM Uganda Research Unit, Immunomodulation and Vaccines Programme
REVIEW RETURNED	17-Mar-2023

GENERAL COMMENTS	It is stated that this will be “a population-representative cross-sectional serosurvey”, how representative is the planned sample of 5,600 of the about 1.34 million Timor-Leste nationals? How have demographic factors such as gender, rural, urban, income levels, and ethnicity been considered in the selection of EAs and households to ensure representativeness? This detail is lacking. Please clarify the proposed age groups, they are stated as 1-4, 5-14, 15-24, 25-40, and >40 years but then in the protocol section on sample size under SARS-CoV-2, it notes the age group of interest as 1-12 years that is not among the proposed age groups. In addition, these age groups are referred to as ‘relevant’ to who, the researchers or the government? Why are non-study team personnel taking part (even if in a passive role) in the study visits e.g. “Municipality Administrator, Sub-Municipality Administrator and/or Head of Community Health Centre, one or two local government representatives and/or one or two Community Health Centre representatives”? These may lead to possible undue influence on the participants. As these are authority figures, their presence may indicate to participants that they have to participate even if they want to or not. E.g. it may seem like a government programme yet this is research! It is noted that the survey will derive a national asset of serum and dried blood spot (DBS) samples, does the consent/ assent provided cater for this? A copy of the information sheet and consent form (and assent form) should be added as well as an appendix to the paper. Will participants with immunosuppressive conditions /treatment e.g. HIV/AIDS or chemotherapy be included in this serology survey? If yes, how will this be catered for while interpreting the results? Why will individuals who report current illness which is compatible with coronavirus disease (COVID-19), be excluded? If it is meant to limit its transmission, what about the asymptomatic who can still transmit but will have no symptoms? If done so will it not limit the study in identifying the extent of local transmission that has occurred? Yet this is one of the intended purposes of the study, please clarify. The plan is to analyze for measles immunoglobulin G (IgG), rubella IgG, severe acute respiratory syndrome coronavirus-2 anti-spike protein IgG, hepatitis B surface antibody and hepatitis B core antigen, how will differentiation be done to know if the cause is an actual infection or the vaccine administered? In the questionnaires to be administered no history of vaccination is being asked. What is the rationale for asking about Dengue in the participant’s questionnaire? It is not listed in table 1 in the vaccination schedule. What appropriate statistical methods will be used to adjust for the performance of the planned serology tests, also what estimates of their sensitivity and specificity are likely to be applicable to the study population?

	No study limitations are stated in the protocol, what are mentioned are risks. One possible limitation is the sample may not be representative of the intended population.

VERSION 1 – AUTHOR RESPONSE

Reviewer: 1
Dr. Wei Wang, Food and Drug Administration Comments to the Author:
In this manuscript, the authors presented study protocol for a population-representative cross-sectional serosurvey to evaluate the Vaccine Preventable Disease Seroprevalence in a Nationwide Assessment of Timor-Leste (VASINA-TL). I mainly focused on the review of the statistical analysis and sample size calculation sections for this protocol based on my expertise. The data analysis presented in this protocol is generally acceptable and I have several comments about the sample size section presented on Page 8 to page 10, specifically,
1) The sample size calculation formula presented in the first line on Page 10 is not correct, the Z value was not included in the formula, please check and correct it.
2) Although the authors listed the non-response rate as 15% in Table 2, I do not believe the non-response rate was considered and used in the sample size calculation, please double check and confirm that.
3) It is not clear why the coefficient of variation was listed in Table 2 as one parameter of the sample size calculation. Please provide explanation about it.
4) It is recommended that the authors presented the sample size calculation formula in a standardized format which is easy to read and understand for the readers, for example, the authors may use the "inserted equation" function provided by Microsoft word to present the sample size calculation formula in the manuscript.

Thank you for these comments. We have revised the sample size description as follows:

"Sample size: A sample size was calculated with reference to the World Health Organization Reference Manual for Vaccination Coverage Cluster Surveys.7 The investigator group considered which age groups would represent the most important sub-categories for estimating vaccine-preventable disease seroprevalence. This process took into account the local vaccine programme history (so serological findings can be related to estimated uptake), existing data on vaccine coverage including the referenced vaccine coverage survey (so that data from each source can be triangulated), programmatic considerations relating to potential intervention(s) in case immunity-gaps are identified, and various logistical and technical considerations (for example excluding individuals <1 year of age because the maternal transfer of antibodies would affect the interpretation and because this age group is more challenging to sample).

VPD seroprevalence estimates which were considered of particular importance to specific age-strata include the following:
- Measles in children under 5 years of age because they are most likely to suffer serious sequelae from infection when compared to other age groups8 and between 5-14 years of age because outbreaks can occur and/or amplify in schools and other settings where groups of children from different households congregate.
- Rubella in women 15-40 years of age because future pregnancies may be at risk of congenital rubella syndrome (CRS). It is anticipated that the rubella virus is circulating in Timor-Leste as there has only been a recent introduction of rubella vaccination and there is very little surveillance for CRS.
- SARS-CoV-2 in children 1-12 years of age because seropositivity is likely to represent naturally acquired infection as this group are not eligible for vaccination in Timor-Leste, which will indicate the extent of local transmission which has occurred.
- Hepatitis B (surface antibody, HBsAb and core antibody, HBcAb) in children under 5 and 5-14 years of age because hepatitis B birth vaccination was introduced approximately 5 years ago and comparison of these groups will give an indication of uptake.

Therefore, each of the following age groups was considered separate strata to provide age-specific seroprevalence results of sufficient precision: 1-4, 5-14, 15-24, 25-40, and 41+ years.

An effective sample size was calculated (i.e. the sample size required if undertaking a simple random sample) of 280 for each stratum. This used the World Health Organization Reference Manual for Vaccination Coverage Cluster Surveys (Table B-1)7 and was based upon an expected seroprevalence of 50% (because this provided the most conservative estimate) and a precision of +/- 6% (because this was precise enough to adequately inform decision-making and small enough to provide a feasible sample size for the financial and human resources available) for the 95% confidence interval.
Without local data on which to confidently base estimates of design effect, a conservative design effect of 4 was estimated, to ensure a sufficient sample to provide precise results. This provided a sample size for each strata of 1120 individuals, and a total survey sample size of 5600.

Based on national census data from 2015 the average number of individuals in each household was 5.7.9 Population projections for 2021 estimated the proportion of individuals in Timor-Leste belonging to each age strata to be 9.6%, 24.0%, 21.9%, 20.2% and 21.5%, respectively. As such, the expected number of required households to sample sufficient individuals from the smallest age strata (1-4 years) was calculated as:

1120 / (0.096 *5.7) = 2047

Household response rate was assumed to be 85%,based on consensus opinion of local investigators who had been involved in previous community surveys in Timor-Leste. The number of households which will be targeted is therefore calculated as:

2047/0.85 = 2408

This lead to 112 EAs being selected (with probability proportionate to municipality population), and 23 households being randomly selected from each EA."

Reviewer: 2
Dr. Ludoviko Zirimenya , MRC/UVRI and LSHTM Uganda Research Unit Comments to the Author:
It is stated that this will be "a population-representative cross-sectional serosurvey", how representative is the planned sample of 5,600 of the about 1.34 million Timor-Leste nationals? How have demographic factors such as gender, rural, urban, income levels, and ethnicity been considered in the selection of EAs and households to ensure representativeness? This detail is lacking.

Thank you for this comment. The survey will be population-representative because the the most recent census data (which enumerated all private households in the country) will be used as the sampling frame, and households will be randomly selected from this, with all household occupants >1year being eligible. We will also collect demographic data from all participants, calculating age/sex-adjusted seroprevalence estimates where appropriate, to account for over/underrepresentation of one section of the population.

Please clarify the proposed age groups, they are stated as 1-4, 5-14, 15-24, 25-40, and >40 years but then in the protocol section on sample size under SARS-CoV-2, it notes the age group of interest as 1-12 years that is not among the proposed age groups.

These age strata were used to calculate sample sizes to ensure adequate precision for these strata. The age group of 1-12 years is of interest for SARS-CoV-2 because of vaccination policy. While this age group was not one of the predefined strata, it is an age group that will include more participants than the 1-4 years stratum, and therefore the precision of seroprevalence estimates for children aged 1-12 years will be greater than that estimated for the 1-4 years stratum.

In addition, these age groups are referred to as 'relevant' to who, the researchers or the government?
This study has been co-designed by investigators at Menzies School of Health Research (Timor-Leste Office), the National Centre for Immunisation Research and Surveillance (Australia), the MoH (Timor-Leste), LNS (Timor-Leste), and the WHO (Timor-Leste Office). Members from this group considered which age groups would represent the most important sub-categories for estimating vaccine-preventable disease seroprevalence. This process took into account the local vaccine programme history (so serological findings can be related to estimated uptake), existing data on

vaccine coverage including the referenced vaccine coverage survey (so that data from each source can be triangulated), programmatic considerations relating to potential intervention(s) in case immunity-gaps are identified, and various logistical and technical considerations (for example excluding individuals <1 year of age because maternal transfer of antibodies would affect interpretation and because this age group is more challenging to sample).

We have added this information to the sample size section.

Why are non-study team personnel taking part (even if in a passive role) in the study visits e.g. "Municipality Administrator, Sub-Municipality Administrator and/or Head of Community Health Centre, one or two local government representatives and/or one or two Community Health Centre representatives"? These may lead to possible undue influence on the participants. As these are authority figures, their presence may indicate to participants that they have to participate even if they want to or not. E.g. it may seem like a government programme yet this is research!

The research will be conducted by Menzies School of Health Research in partnership with the Ministry of Health. Ministry of Health involvement in and leadership of research with public health importance is not unusual in Timor-Leste. Presence of these authority figures will indicate permission and approval for the research to take place, in a setting where people would be reluctant to participate in research without such assurance. However, we also acknowledge the importance of ensuring that potential participants are aware that they do not have to participate, and that they can say no. Field teams will be trained in Good Clinical Practice (GCP) and when seeking informed consent, will emphasise that potential participants are under no obligation to participate. Government officials will also be briefed, so that they understand the approach to obtaining informed consent, and ensure hat there is no undue influence or coercion, so that they can emphasise this in their communication with community-members as well. This approach is consistent with previous community-based research we have conducted in Timor-Leste.

It is noted that the survey will derive a national asset of serum and dried blood spot (DBS) samples, does the consent/ assent provided cater for this? A copy of the information sheet and consent form (and assent form) should be added as well as an appendix to the paper.

Yes, this information is in the participant information sheet. A passage in the consent form reads as follows:

The anonymised blood sample and 'dried blood spot' being stored for 10 years and being used to validate tests for infections, and potentially tested for other communicable or non-communicable diseases

Participant information sheets and consent forms are now included as appendices

Will participants with immunosuppressive conditions /treatment e.g. HIV/AIDS or chemotherapy be included in this serology survey? If yes, how will this be catered for while interpreting the results?

This survey is likely to include individuals who have immunosuppressive conditions because it is intended to be population-representative (>1 year of age) and has broad inclusion criteria. However, the survey will not collect detailed clinical information from individual participants (apart from that which is specifically related to vaccine uptake). This decision was taken because the investigator group felt that conducting prolonged interview questionnaires including potentially sensitive health information may make some potential participants uncomfortable. Therefore, ease-of-questionnaire-administration and survey acceptability +/- uptake was prioritised.

We understand that interpreting serology and using data to infer estimates of population immunity and/or vaccine uptake in this group can be challenging. However, we estimate that the prevalence of immunosuppressive conditions in Timor-Leste to be low and therefore consider this to be a minor limitation to our study. Prevalence of HIV in Timor-Leste is estimated to be 0.2% in those aged 15-49 years. Availability of chemotherapy for malignant conditions, with the exception of corticosteroids, is limited.

Why will individuals who report current illness which is compatible with coronavirus disease (COVID-19), be excluded? If it is meant to limit its transmission, what about the asymptomatic who can still transmit but will have no symptoms? If done so will it not limit the study in identifying the extent of local transmission that has occurred? Yet this is one of the intended purposes of the study, please clarify.

This exclusion criterion has been included as risk-mitigation for field teams. Universal precautions against the potential transfer of infectious disease between participants and field teams (in either direction) will also be used. These include using gloves, masks, and aprons during all participant-facing activities (including during interviews) and standard precautions against blood-borne viruses (during phlebotomy and/or finger-prick procedures). Standard operating procedures and training for fieldworks which include these details were developed in accordance with relevant local and international guidance and will be reviewed +/- updated throughout the study.

It is possible that exclusion of individuals with current illness compatible with COVID-19 will lead to an underestimation of seroprevalence. However, since acute illness lasts <2 weeks for most people, yet anti-S seropositivity typically lasts for many months, we believe this effect will be low. However, the impact depends on the timing of fieldwork in relation to any local outbreaks of SARS-CoV-2 (i.e. the prevalence of acute infection at the time of survey), which is difficult to predict.

The plan is to analyze for measles immunoglobulin G (IgG), rubella IgG, severe acute respiratory syndrome coronavirus-2 anti-spike protein IgG, hepatitis B surface antibody and hepatitis B core antigen, how will differentiation be done to know if the cause is an actual infection or the vaccine administered? In the questionnaires to be administered no history of vaccination is being asked.

For hepatitis B we will test samples for antibodies against surface antigen and core antigen, which will help differentiate vaccine response from infection.

For SARS-CoV-2 a decision has been made to test for anti-S (but not anti-N) antibodies for three main reasons. First, the most important seroprevalence information is felt to be that which is most associated with population immunity (i.e. that which may be 'correlated with protection'), regardless of whether this immunity is vaccine- or infection-derived. Second, whole-virus vaccines may be used in Timor-Leste, which cannot be differentiated from such methods. Third, since we elected to use commercially available assays at NHL, there would be a significant cost associated with analysing 5600 samples for another target.

For measles and rubella, differentiation of vaccine- and infection-derived immunity is challenging because vaccines are live-attenuated.

What is the rationale for asking about Dengue in the participant's questionnaire? It is not listed in table 1 in the vaccination schedule.

This is because dengue is a significant cause of morbidity and mortality in Timor-Leste and a major priority for MoH, WHO and local researchers. Potential forthcoming regional and/or national disease control interventions include the roll-out of existing (Dengvaxia®) or novel (Takeda, pending trial data) vaccines and the deployment of Wolbachia mosquitos and it is possible that samples may be used in baseline studies of seroprevalence (i.e. regional estimation of dengue force-of-infection).

What appropriate statistical methods will be used to adjust for the performance of the planned serology tests, also what estimates of their sensitivity and specificity are likely to be applicable to the study population?

This is an important consideration. The cited performance of serological assays depends on what is used as a reference standard.

This might be based on results from another (previously validated) assay which has been used in parallel. If we adjust our results based on this type of data, we will end up with a set of results which indicate what the seroprevalence in our population 'would have been', had we used that alternative assay. This may be a useful exercise. For example, we may analyse a subset of our samples at an

international reference laboratory. If there is significant variation in results between assays we would adjust findings accordingly (assuming that the reference laboratory has a more accurate assay).

Alternatively, assay performance might be assessed 'de-novo', using a panel of samples from individuals who have either been infected vaccinated previously. If this panel is well characterised and is relatable to our population (in terms of demographic variables and crucially the timing of infection/vaccination), then we would consider adjusting our findings according to those data. This may allow us to make more accurate estimates of vaccine uptake and/or number previously infected. A literature review will be conducted at the time of reporting, to make this decision.

However, we consider the most informative 'end-point' in our study to be that of 'population immunity' (i.e. asking the question 'what proportion remain susceptible to infection?'). This is why we looked to the literature on 'correlates of protection' and as far as possible have chosen validated assays which give a quantitative determination of antibody concentration. In these cases we will not adjust based on estimates of assay sensitivity/specificity unless data emerge which suggest a new correlate of protection should be used (and that the assay we have used has suboptimal performance in determining this).

No study limitations are stated in the protocol, what are mentioned are risks. One possible limitation is the sample may not be representative of the intended population.

We have added significant limitations, including those you kindly point out in your earlier comments

Reviewer: 1
Competing interests of Reviewer: I have no conflicts of interest.

Reviewer: 2
Competing interests of Reviewer: None

VERSION 2 – REVIEW

REVIEWER	Wang, Wei Food and Drug Administration, Division of Biostatistics, Center for Devices and Radiological Health
REVIEW RETURNED	18-Apr-2023

GENERAL COMMENTS	Although the authors did not follow originally proposed sample size calculation presentation format, the updated sample size calculation section is correct, and I have no further comments.

REVIEWER	Zirimenya , Ludoviko MRC/UVRI and LSHTM Uganda Research Unit, Immunomodulation and Vaccines Programme
REVIEW RETURNED	16-Apr-2023

GENERAL COMMENTS	The authors have satisfactorily addressed all comments.

VERSION 2 – AUTHOR RESPONSE