PEER REVIEW HISTORY

ARTICLE DETAILS

Title (Provisional)

Prevalence of syphilis infection among migrant workers in Qatar: a nationwide cross-sectional survey

Authors

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VERSION 1 - REVIEW

Reviewer	**1**
Name	**Konda, Kelika**
Affiliation	**Universidad Peruana Cayetano Heredia, School of Public Health and Administration**
Date	**30-Jan-2024**
COI	**I have no competing interests.**

The premise of the study is important and the population used and methods generally make sense for the study goals. However, the focus on lifetime syphilis prevalence is problematic, although unfortunately the only available route given the data. There is no information on how long the individuals have been in Qatar, so their infection with TP at some point in their life is not what is needed for Qatari public health policy. While the prevalence encountered

is helpful as a baseline and the authors call for additional services for the target population makes sense, they should assure that the discussion of the findings calls attention to the need for sexual and reproductive health services. This does not appear to be a population at high risk of STIs.

The acronym CMW oddly doesn't point out that the individuals under study are expatriates. I realize that Qatari society is almost all expatriates and that this acronym may be commonly used there, but for an international audience 'craft and manual workers' doesn't imply expatriates or migrants. The title of the paper mentions 'migrants', but this term is not used in the text to describe the population under study – it is only used in the introduction to point out the need for STI data on migrants. I would suggest using a term that matches the title and also indicates that this group are all expatiates or migrants.

While it is likely that an RPR ≥1:8 is indicative of recent infection, unfortunately the reverse isn't true for RPR<1:8. While often these RPRs can signal past infection, there may be misclassification of low RPRs that actually are recent (within the past year). I would suggest sticking to the terms recent infection and lifetime infection, the difference between these as "remote" infection isn't necessarily accurate and given that recent is a subset of lifetime the subtraction to get to the other group isn't needed.

The authors do a good job of detailing the limitations of their syphilis diagnostic strategy in the discussion section, but should assure that the methods and results match their data, i.e. avoid "remote" infection, lifetime and recent are both accurate.

The abstract doesn't make clear the cutoff of RPR ≥1:8 as the indicator of recent infection. The abstract also doesn't mention the weighting used in the methods. This means that in the abstract, the percentages and their 95% CIs appear to be incorrect. The reader needs more information on the methods to understand the calculations presented.

In the methods section the weighting is mentioned at the outset and specifically stated as having been conducted to adjust the prevalence of lifetime and recent syphilis. This section does not clarify if weighting was used for the logistic regression model. This should be clear to the reader and if weighting was not used for the regression – why not?, given that the authors appear to believe it was necessary to control for sampling frame. The multiple imputation used to address the missing data point is stated in the same sentence as the weighting for the prevalence of lifetime and recent, but its for a different purpose (missing data) – was this done only for the prevalence estimates? Or was the resulting imputed dataset also used for the logistic regression?

Univariate logistic regression would only make sense if only the outcome were included. The authors state that this was used to study associations with lifetime syphilis, that implies bivariate regression (i.e. an independent and a dependent variable).

Given that the testing was done on Covid remnant samples, is it possible that there was an association between positivity for sars-cov-2 and TP infection that influence the prevalence as those samples were unavailable for testing?

The authors do not have information on the sex lives of the CMW population, so the conclusion that “The observed prevalence of syphilis indicates active transmission of this infection within the CMW population” is unfounded. The authors state in the next sentences that the sexual networks of the CMW population are unknown. But the first sentence in that paragraph needs to be modified. The prevalence of syphilis is an indication of sex (a normal human behavior) not active transmission within the CMW population. The authors go on to say that their inference about syphilis is bolstered by the prevalence of herpes that they found among the same population; however, the prevalence included in their article (11%) is equivalent to the prevalence of HSV-2 in Europe and about half the prevalence found in the United States. While documenting the prevalence of STIs and calling for health care to address STIs are important goals, it does not appear that either prevalence, syphilis or HSV-2, is alarmingly high and the authors should reconsider the wording of this paragraph.

The separation between the two paragraphs on limitations related to syphilis testing should be eliminated.

Reviewer	**2**
Name	**Kamb, Mary**
Affiliation	**U. S. Centers for Disease Control and Prevention , Division of STD Prevention**
Date	**07-Feb-2024**
COI	**No competing interests**

This is a secondary analysis of data from a COVID-19 survey among migrant workers in Qatar that assesses syphilis prevalence (lifetime and active infection). I found research question appropriately addressed and the paper well organized and written. I thought the statistical methods looked appropriate although am not entirely certain about the procedures around use of weighted estimates given unequal sampling of participants. See one suggestion on Methods below.

Regarding findings, the 1.3% lifetime infection is not unanticipated (actually might be low compared with some other global estimates), but since data from this population are not previously known I think these are good data to have in published literature.

Minor suggestions for the authors are to report in Methods, if possible, how RPR testing was done and any quality control/quality assurance procedures done for RPR in their lab (a somewhat subjective test). Also, although a positive treponemal test usually represents a

past syphilis infection, other treponemal conditions (e.g., yaws in childhood) could cause positivity; yaws was prevalent in the past in some of the countries of origin for some migrants. This might be relevant to note in limitations.

VERSION 1 - AUTHOR RESPONSE

Prevalence of syphilis infection among migrant workers in Qatar: a nationwide cross-sectional survey

REPLY TO EDITORIAL AND REVIEWERS' COMMENTS

We are grateful to the editors and reviewers for assessing our work and for their insightful and useful feedback and suggestions. Please find below a point-by-point reply addressing each of the comments. We have also incorporated these suggestions in the revised manuscript, as noted below. We would be pleased to address any additional matters, should that be necessary.

Note: All references to the revised manuscript pertain to the marked copies of the manuscript files including changes implemented through "track changes".

Reviewer(s)' Comments to Author:

Reviewer: 1

Dr. Kelika Konda, Universidad Peruana Cayetano Heredia, University of California Los Angeles David Geffen School of Medicine

Comments to the Author:

Comment: We thank the reviewer for the time and effort put into this review, the assessment of our work, and the constructive feedback on our manuscript that enriched it and improved its readability. Please find below a point-by-point reply addressing each of the reviewer's comments.

The premise of the study is important and the population used and methods generally make sense for the study goals. However, the focus on lifetime syphilis prevalence is problematic, although unfortunately the only available route given the data. There is no information on how long the individuals have been in Qatar, so their infection with TP at some point in their life is not what is needed for Qatari public health policy. While the prevalence encountered is helpful as a baseline and the authors call for additional services for the target population makes sense, they should assure that the discussion of the findings calls attention to the need for sexual and reproductive health services. This does not appear to be a population at high risk of STIs.

Answer: Thank you for the useful insights and suggestions. We agree with the reviewer that the nature of the study sample limits the scope of what could be done and generated in this

study. Unfortunately, we do not have access to data on how long these individuals have been in Qatar. While our focus was on lifetime syphilis prevalence, we also reported results for recent infection, which is an important marker of recent acquisition. As the reviewer indicated, the study findings underscore the need for enhanced efforts in STI prevention and treatment, particularly in such settings where such programs are inadequate.

To address the reviewer's comment, we have expanded the discussion to emphasize the need for sexual health services (Discussion, Page 14, Paragraphs 2 and 3). Additionally, we have highlighted the limitation of not having data on the duration of residency in Qatar, which reduces the relevance of the lifetime infection information for informing public health policy in Qatar (Discussion, Page 17, Paragraph 1).

The acronym CMW oddly doesn't point out that the individuals under study are expatriates. I realize that Qatari society is almost all expatriates and that this acronym may be commonly used there, but for an international audience 'craft and manual workers' doesn't imply expatriates or migrants. The title of the paper mentions 'migrants', but this term is not used in the text to describe the population under study – it is only used in the introduction to point out the need for STI data on migrants. I would suggest using a term that matches the title and also indicates that this group are all expatiates or migrants.

Answer: Thank you for the useful suggestions. The acronym has now been changed from CMW to MCMW (migrant craft and manual worker) (multiple instances throughout the manuscript). Additionally, the population has been explicitly defined as expatriate and migrant (Abstract and Introduction, Page 6, Paragraph 3 and Page 7, Paragraph 1).

While it is likely that an RPR ≥1:8 is indicative of recent infection, unfortunately the reverse isn't true for RPR<1:8. While often these RPRs can signal past infection, there may be misclassification of low RPRs that actually are recent (within the past year). I would suggest sticking to the terms recent infection and lifetime infection, the difference between these as "remote" infection isn't necessarily accurate and given that recent is a subset of lifetime the subtraction to get to the other group isn't needed.

Answer: As the reviewer will appreciate, syphilis diagnosis is inherently complex due to the lack of precise and direct diagnostic tools for current infection, making it challenging to establish clear-cut criteria. However, we appreciate the reviewer's point and the benefits of simplifying the interpretation. Consequently, we have removed all mentions and results of remote infection throughout the manuscript, focusing exclusively on lifetime versus recent infection, as suggested (multiple instances throughout the manuscript).

The authors do a good job of detailing the limitations of their syphilis diagnostic strategy in the discussion section, but should assure that the methods and results match their data, i.e. avoid "remote" infection, lifetime and recent are both accurate.

Answer: Thank you for the useful suggestion. As indicated in our response above, we have removed all mentions and results of remote infection throughout the manuscript, focusing exclusively on lifetime versus recent infection, as suggested (multiple instances throughout the manuscript).

The abstract doesn't make clear the cutoff of RPR ≥1:8 as the indicator of recent infection. The abstract also doesn't mention the weighting used in the methods. This means that in the

abstract, the percentages and their 95% CIs appear to be incorrect. The reader needs more information on the methods to understand the calculations presented.

Answer: Excellent points, thank you. The abstract has been revised to clarify that an RPR titer of ≥1:8 is the indicator of recent infection and to indicate the use of weighting in the methods (Abstract).

In the methods section the weighting is mentioned at the outset and specifically stated as having been conducted to adjust the prevalence of lifetime and recent syphilis. This section does not clarify if weighting was used for the logistic regression model. This should be clear to the reader and if weighting was not used for the regression – why not?, given that the authors appear to believe it was necessary to control for sampling frame. The multiple imputation used to address the missing data point is stated in the same sentence as the weighting for the prevalence of lifetime and recent, but its for a different purpose (missing data) – was this done only for the prevalence estimates? Or was the resulting imputed dataset also used for the logistic regression?

Answer: We apologize for the confusion. Sampling probability weights were applied to all statistical analyses, including the estimation of recent and lifetime syphilis infection prevalence and logistic regression analyses exploring associations with lifetime syphilis infection. Multiple imputation was performed to predict the recent infection status of the single treponemal-positive specimen that had adequate sera for *T. pallidum* antibody testing using the Mindray CL-900i Chemiluminescence Immunoassay Analyzer but lacked sufficient sera for RPR testing. The imputed dataset was used solely to estimate the proportions of recent syphilis infections. No additional regression analyses were conducted to explore associations with recent infection due to the limited number of recent syphilis cases.

We have now substantially revised this section of the methods to clarify these points (Methods, Page 10, Paragraphs 2-4 and Page 11, Paragraph 1).

Univariate logistic regression would only make sense if only the outcome were included. The authors state that this was used to study associations with lifetime syphilis, that implies bivariate regression (i.e. an independent and a dependent variable).

Answer: We appreciate the reviewer's feedback. We have now revised the terminology to "bivariable analysis" (Methods, Page 11, Paragraph 1, and Table 2).

Given that the testing was done on Covid remnant samples, is it possible that there was an association between positivity for sars-cov-2 and TP infection that influence the prevalence as those samples were unavailable for testing?

Answer: To clarify any potential misunderstanding, please note that the tested specimens were collected during a nationwide cross-sectional survey to estimate SARS-CoV-2 seroprevalence.[1] Therefore, the sample includes both SARS-CoV-2 seropositive and seronegative specimens. This point has been clarified (Abstract and Methods, Page 7, Paragraph 2).

It is acknowledged that the possibility cannot be excluded that, for some assays, SARS-CoV-2 seropositivity may impact RPR testing outcomes.[2] However, our study does not have the appropriate design to explore this question. Additionally, considering the different modes of

transmission, it seems epidemiologically unlikely that there is an association between SARS-CoV-2 positivity and syphilis positivity. This was confirmed by the logistic regression analysis, which indicated an OR of 1.30 (95% CI: 0.64-2.64) for lifetime syphilis infection in SARS-CoV-2 seropositive individuals compared to SARS-CoV-2 seronegative individuals. Even if an association were observed, interpretation would be problematic as it might reflect a confounding factor rather than a true biological relationship. To avoid confusion or misleading interpretations, this measure of association is not included in our analyses. Nevertheless, we indicated that it remains unclear whether SARS-CoV-2 antibody positivity could affect the outcome of RPR testing for the specific assay utilized in this study (Discussion, Page 15, Paragraph 4 and Page 16, Paragraph 1).

The authors do not have information on the sex lives of the CMW population, so the conclusion that "The observed prevalence of syphilis indicates active transmission of this infection within the CMW population" is unfounded. The authors state in the next sentences that the sexual networks of the CMW population are unknown. But the first sentence in that paragraph needs to be modified. The prevalence of syphilis is an indication of sex (a normal human behavior) not active transmission within the CMW population. The authors go on to say that their inference about syphilis is bolstered by the prevalence of herpes that they found among the same population; however, the prevalence included in their article (11%) is equivalent to the prevalence of HSV-2 in Europe and about half the prevalence found in the United States. While documenting the prevalence of STIs and calling for health care to address STIs are important goals, it does not appear that either prevalence, syphilis or HSV-2, is alarmingly high and the authors should reconsider the wording of this paragraph.

Answer: Thank you for the useful points. To address the reviewer's comment, we have substantially revised this paragraph, factoring the caveats raised by the reviewer (Discussion, Page 14, Paragraph 2).

The separation between the two paragraphs on limitations related to syphilis testing should be eliminated.

Answer: We appreciate the reviewer's point. However, we have now added a third paragraph addressing also limitations of syphilis testing. Merging all these points into a single paragraph would result in an overly long paragraph, which may be difficult to read and follow, especially given the subtlety of the indicated points.

Reviewer: 2

Dr. Mary Kamb, U. S. Centers for Disease Control and Prevention

Comments to the Author:

This is a secondary analysis of data from a COVID-19 survey among migrant workers in Qatar that assesses syphilis prevalence (lifetime and active infection). I found research question appropriately addressed and the paper well organized and written. I thought the statistical methods looked appropriate although am not entirely certain about the procedures around use of weighted estimates given unequal sampling of participants. See one suggestion on Methods below.

Regarding findings, the 1.3% lifetime infection is not unanticipated (actually might be low

compared with some other global estimates), but since data from this population are not previously known I think these are good data to have in published literature.

Comment: We thank the reviewer for the time and effort put into this review, the assessment of our work, and the constructive feedback on our manuscript that enriched it and improved its readability. Please find below a point-by-point reply addressing each of the reviewer's comments.

Regarding the sampling probability weights and their incorporation into the analyses, this section of the methods has been substantially revised to clarify these points (Methods, Page 10, Paragraphs 2-4 and Page 11, Paragraph 1). Additionally, the methods part of the abstract has been revised to provide clarity on these points (Abstract).

Minor suggestions for the authors are to report in Methods, if possible, how RPR testing was done and any quality control/quality assurance procedures done for RPR in their lab (a somewhat subjective test).

Answer: Excellent suggestion, thank you. Detailed methods for the RPR testing and the quality control/quality assurance procedures have now been added as a section in the Supplemental Material (Section S1 in Supplemental Material) and cited in the main text (Methods, Page 9, Paragraph 3).

Also, although a positive treponemal test usually represents a past syphilis infection, other treponemal conditions (e.g., yaws in childhood) could cause positivity; yaws was prevalent in the past in some of the countries of origin for some migrants. This might be relevant to note in limitations.

Answer: Excellent point, thank you. We have now discussed this point in the limitations (Discussion, Page 15, Paragraph 4).

Reviewer: 1
Competing interests of Reviewer: I have no competing interests.

Reviewer: 2
Competing interests of Reviewer: No competing interests

References

1. Al-Thani MH, Farag E, Bertollini R, et al. SARS-CoV-2 Infection Is at Herd Immunity in the Majority Segment of the Population of Qatar. *Open Forum Infect Dis* 2021;8(8):ofab221. doi: 10.1093/ofid/ofab221 [published Online First: 2021/08/31]
2. Xiong-Hang K, MacLennan A, Love S, et al. Evaluation of False Positive RPR Results and the Impact of SARS-CoV-2 Vaccination in a Clinical Population with a High Rate of Syphilis Utilizing the Traditional Screening Algorithm. *American Journal of Clinical Pathology* 2022;158(Supplement_1):S17-S18. doi: 10.1093/ajcp/aqac126.030

VERSION 2 - REVIEW

Reviewer	**2**
Name	**Kamb, Mary**
Affiliation	**U. S. Centers for Disease Control and Prevention , Division of STD Prevention**
Date	**05-Oct-2024**
COI	

Overall, thought this version of the paper looked good. I have few specific suggestions. However, reference 26 is a little odd -- there are standard interpretations of these tests that do not require personal communications as a reference (and I see Dr. Klausner is acknowledged in any case). For test interpretation, might consider referencing one of the more in-depth papers on syphilis that go into interpretation of diagnostic tests (e.g., Peeling RA et al. Syphilis in Nature Reviews Disease Primers, 2017).