Author’s response to reviews

Title: Risk factors for viral hepatitis C infection in Rwanda: Results from a Nationwide Screening Program

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Version: 1 Date: 05 Jul 2019

Author’s response to reviews:

July 5th, 2019

Dear Amy Matser

BMC Infectious Diseases

Enclosed is a revised version of our manuscript, titled: "Risk factors for viral hepatitis C infection in Rwanda: Results from a Nationwide Screening Program" (INFD-D-19-00889) for your renewed consideration as an original contribution to the Journal.
Thank you for the opportunity to improve our manuscript by responding to the comments of the reviewers. We believe that the manuscript has now been substantially improved. Below we respond (italics) to specific comments and queries of the reviewers (bold).

We look forward to your response.

Yours sincerely,

Jean Damascene Makuza, MD, MSc

General important aspects:

- The exclusion of HCV RNA+ individuals should be discussed. If these individuals are excluded from the study (not only from testing), you are investigating the prevalence and factors associated with undiagnosed HCV. The overall HCV prevalence is underestimated, because this is the sum of known and undiagnosed infections. This should be mentioned in the discussion.

Response: Thank you for the comments, the exclusion of individual who were aware that are HCV RNA+ prior the screening could cause a selection bias. However, we did not find any single such case during screening. Thus, in order to address your comment, we changed the exclusion criteria.

- The multivariable model was obtained using a backward stepwise regression according to the methods, but the multivariable model displayed in table 3 still includes all, also non-significantly associated variables (p>=0.05). I recommend to perform a backward selection procedure and remove those variables that are not significantly associated.

Response: Thank you for the comments, it was corrected in this new version, all variables with non-significant association in multivariable analysis were removed.

- Some 'obvious' risk factors or routes of transmission, such as injecting drug use and sexual contact between men who have sex with men are not included or discussed in the article. If data on these transmission routes are not available, this is a limitation which should be discussed. When these factors are known to be unrelated to the HCV epidemic in Rwanda this should be discussed as well.

Response: Thank you for the comments, this study was done on data collected during screening of general population in Rwanda. The probability of having IDU and MSM during this study was very low due to fact those are still hidden population and very small number in Rwanda, referring to WHO and UNAIDS estimates of IDU and MSM. However, it is a limitation thus for next studies we will target IDU and MSM.
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Reviewer reports:

Reviewer #1: Giuseppina Ortu, PhD (Reviewer 1): Please include all comments for the authors in this box rather than uploading your report as an attachment. Please only upload as attachments annotated versions of manuscripts, graphs, supporting materials or other aspects of your report which cannot be included in a text format. Please overwrite this text when adding your comments to the authors.

Review of the manuscript:

The paper is relevant although I after reading I felt it was to some extent a missed opportunity to know a bit more in details about risk factors in Rwanda. I also have some doubts about the analysis.

Major concern: survey sampling

The manuscript states:

The total number of individuals screened was 327,383; 326,263 (99.7%) had available of which 22,183 (6.8%) screened positive for HCVAb

Response: Thank you for the comments, we clarified in updated version that 326,263 (99.7%) are people who received their results as other 0.3% did not receive them due to invalid tests, lack of samples and other errors which could happen in testing.

The MoH did the screening and people voluntarily accepted to be screened as they knew they would have received free screening and treatment. 6.8% of these screened people resulted positive to the antibody. This positive population should have been the population analyzed and compared to the remaining population that was negative to the antibody.

It seems that the risk assessment analysis was done in the whole screened population (e.g. see table on self-reported risk factors) – why wouldn’t the authors consider to perform the risk analysis in those HCVAb positive only?

I would highly suggest redoing the analysis only considering this sub-set of people, those positive, and see how the results change, and how comparable they are with those people found negative. Also, the tables should reflect this (e.g. tables with descriptive stats for the whole
group, table with descriptive stats for the negative one and tables for the positive ones and then a table highlighting the comparison).

Response: Thank you for the comments, we did correction for descriptive analysis and considered descriptive stats for the whole group, for negative cases and for positive cases alone. The table highlighting the comparison is the analytical one below the two cited above. The risk factors were assessed on those who tested HCVAb positive.

Ubudehe category –

It would be suggested to add more details about this socioeconomic status/category – What type of income? What are exactly these categories? What type of occupation, if there is any link of a category to an occupation? Some more details would maybe provide better insight into the reasons why certain categories are more at risk.

Response: Thank you for the comments, we did addition of more details on Ubudehe Category in the manuscript in method-variables. Details added in reported below ‘The households that consume less than Rwandan Francs (RWF) 105,064 [equivalent to USD 120] per year are classified in Ubudehe Category 1 (poorest category), which includes people without houses and those affected by food insecurity. Households that consume between RWF 105,064 and RWF 159,375 (exclusively) [equivalent to USD 180] per year are in Ubudehe Category 2, which includes households subsisting on manual labor or temporary employment and those capable of renting or owning their own houses. Categories 1 and 2 receive government assistance. Households that consume more than or equal to RWF 159,375 are classified in Ubudehe Category 3 and 416. These categories are not considered poor and do not receive government assistance. Category 3 includes wealthy farmers and investors while Category 4 includes large business owners and highly ranked government officials17.’

Risk factors

Traditional surgical operations: it would have been useful to list them all in the questionnaire. I have the feeling that many people may not have called traditional operations as such and may have not reported them if not explicitly listed in the questionnaire – even the manuscript does not list them all. For instance, the first time they have been mentioned, male circumcision is not listed among them, while it is mentioned in the discussion – this is a missed opportunity as if the main link was male circumcision, then the type of community sensitization would be very different from, for instance, dental extraction. So it seems that this survey has to be redone to collect much more detailed information….

Response: Thank you for the comments, I agree with you that is a missing opportunity. However, before starting the screening trained health care providers had to explain all traditional surgical operations to all participants one by one as they were trained before on that. In addition to that some of them were listed in questionnaire in maternal tongue: Kinyarwanda (ibyinyo=dental extraction, indasago=traditional minor surgery, imyotso=scarifications, ibirimi=uvulectomy,
…). In manuscript also we incorporated all in variables of the updated version including male circumcision not done at a health facility. ‘Traditional surgical operation practices are defined as scarifications, male circumcision, tattoos, traditional dental extraction or uvulectomy done by a community member or traditional practitioner.’

Occupational risk

This is a significant issue - why did the authors not add it to the information collected during the survey? It is again a missed opportunity – this information could have provided some important information about occupational risk and could have led to some policies to tackle this problem. The authors should state why this question was not asked. It is not possible to take for granted that medical staff in health care facilities is not at risk, for instance, and it would have been good to have a list of occupation that may put at risk either the service provider or the consumer …

Response: Thank you for the comments, I agree with you that this is a missing opportunity and was put in limitations. We did not collect this information as we had some studies which were performed in health care providers and find low prevalence among them. However, for next studies we will consider them in order to know its unknown contribution to HCV risk factors.

‘Other unmeasured risk factors such as exposure to mass casualties through war and conflict, sexual violence, refugee status, occupational risk like health care providers or community based traditional practices may have been more prevalent historically’.

Other risk factors

Also, the same-sex partner could have been another type of question or drug use – perhaps these are not relevant issues in Rwanda, but if not then the authors should again state why they did not ask these questions.

Response: Thank you for the comments, this study was done on data collected during screening of general population in Rwanda. The probability of having IDU and MSM during this study was very low due to fact those are still hidden population and very small number in Rwanda, referring to WHO and UNAIDS estimates of IDU and MSM. However, we put them in limitation and for next studies we will consider them in order to know its unknown contribution to HCV risk factors.

Other issues

The manuscript needs some minor editing, even the supplementary information (but I am not the best person to highlight where editing is needed as English is not my first language).

Response: The text was reviewed and edited by the native English speakers (NG, MH and CL).
Reviewer #2 Anne Oevrehus:

Major comments

Very large and impressive study finding a high seroprevalence among people in Rwanda.

Important data but how well do they reflect the Rwanda population is my major concern

It should be more clearly described if the tested population (69% women? And persons< 25 years excluded?) is representative for Rwanda with regards to the most important characteristics (sex, age distribution, HIV sero prevalence, socioeconomic, geographical). Is there a national census that can provide insight?

Response: Thank you for the comments, this study was done on data collected during screening of general population in Rwanda. One of its limitations was lack of representativity for different factors including sex, age distribution, HIV seroprevalence, socio-economic status and geographical area. This was discussed in limitation ‘The demographic profile of the sample population of voluntary participants differed from the Rwandan population at large, with a substantially greater proportion of females (69%) and a higher median age (43.0) than the general population. Thus, the prevalence estimate and risk factors found to be associated with HCVAb may not be generalizable to the entire population.

It should also be more clear how the participants in the study were enrolled and what bias this might have caused.

Response: Thank you for your good comments, how study participants were enrolled and bias this might cause was clarified in the manuscript.

‘The sample population consisted of voluntary participants who are likely to differ in risk profile compared to the Rwandan population at large. Thus, the prevalence estimate and risk factors found to be associated with HCVAb may not be generalizable to the entire population’.

People who inject drugs(PWID) are not mentioned. It might be that it is not an issue but for the international reader any considerations about omitting this in most context important route of transmission should be described

Response: Thank you for the comments, this study was done on data collected during screening of general population in Rwanda. The probability of having IDU and MSM during this study was very low due to fact those are still hidden population and very small number in Rwanda, referring to WHO and UNAIDS estimates of IDU and MSM. However, for next studies we will consider them in order to know its unknown contribution to HCV risk factors.
In the multi variable analysis consider including fewer variables or merging some of the categorical variables with very few in each strata and examine for interactions between variables that might moderate effect.

Response: Thank you for the comments, categorical variables with very few number were merged with others like Ubudehe category 4 which were merged with category 3

Minor comments

Abstract

Clear and well written

Results. Ubudehe category and RAMA insurance are not a commonly known variables - please rephrase (socioeconomic category / national health insurance?)

Response: Thank you for the comments, in updated version they have been defined and rephrased in the manuscript. “Ubudehe is a development programme whereby citizens are placed into different categories. The lowest socioeconomic category is 1 and the highest is 4. Socioeconomic status was defined in accordance with the updated Ubudehe category by the Ministry of Local government of Rwanda (MINALOC)15”; “For health insurance, only community-based health insurance (Mutuelle), la Rwandaise Assurance Maladie (RAMA) which is Health insurances for employees of public and private sectors”.

Maybe a matter of taste but three-digit decimal are not needed for the OR - one digit or two at the most would be sufficient

Response: Thank you for the comments, in updated version we considered only 2 digits for OR

Introduction

Nicely outlines why the study is needed

Line 55-57

Consider rephrasing - sounds like health workers have recreational exposure to blood?

Response: Thank you for the comments, in updated version we rephrased the sentence

Page 2 line 4-8

Is injecting drug use not a risk factor for transmission in Rwanda?
Response: Thank you for the comments, this study was done on data collected during screening of general population in Rwanda. The probability of having IDU and MSM during this study was very low due to fact those are still hidden population and very small number in Rwanda, referring to WHO and UNAIDS estimates of IDU and MSM. However, for next studies we will consider them in order to know its unknown contribution to HCV risk factors.

Study design

Please elaborate on the design - what was included in the standardized laboratory request form and how has it completed?

Response: Thank you for the comments. The contents of the standardized laboratory request form were added in the test of study design in summary but developed in variables. ‘All data used for analysis was collected at the time of viral hepatitis screening through use of a standardized laboratory request form which contained sociodemographic, comorbidities and viral hepatitis risk factors characteristics’.

Study population

What was the rationale for excluding people < 25 years of age - this is likely to have a major impact on results and make national adult sero-prevalence less comparable to other studies?

Response: Due to the likely lower prevalence in those under 25 years old, only older individuals were screened for this study. We included this in the limitations paragraph of the Discussion section.

Was screening offered everyone in the community or only those presenting with a wish for test. (testing bias)

Response: We only offered only those with a will after sensitization and we acknowledge volunteer bias. We added this information to the limitations paragraph in the discussion.

Persons known to HCV-RNA + were excluded from the study or from the testing? Important with regards to Data collection

Response: Thank you for the comments, this is a missed opportunity but it was corrected and changed it the manuscript as we did not find anyone with HCV RNA+ during analysis

Did the risk factors include intravenous drug use? Please clarify
Response: Thank you for the comments, as stated above this study was done on data collected during screening of general population in Rwanda. In general, according to WHO, Rwanda has non-significant number of People who inject drugs and its consequences are not a public health problem therefore we did not consider it in this study. However, for next studies we will consider them in order to know its unknown contribution to HCV risk factors.

Was the anti HCV test validated in the target population to ensure performance? Any validation of corresponding HCV-RNA?

Response: We used Enzyme Linked Immuno-Sorbent Assay(ELISA) test which is internationally accredited by WHO but before using it also Rwanda National Referral Laboratory with the HIV, STIs and OBBI Division in a Viral Hepatitis Technical Working Group (TWG) validated this using HCV-RNA.

Variables included in the analysis are mentioned both in the data collection section and variables section which is a little confusing should preferably be in the same section.

Response: Thank you for the comments, it was corrected in this new version

Were the data collected before or after the participant received the result of the test

Response: Data were collected before participants received the results of the test as cited in study design: ‘All data used for analysis were collected at the time of viral hepatitis screening through use of a standardized laboratory request form’.

Having one more sexual partner asked as ever or currently? (seems fairly unlikely that 97.3 had only had one partner ever?)

Response: Thank you for the comments, it was as ever. Because it was a self-reporting, we could face some social desirability bias as reported in limitations. Having 97.3% of participants who had only one partner ever should be a bias.

Statistical method

Using logistic regression to assess determinants for HCV exposure is fair but as mentioned in the Major comments section and below consider to

Line 53-53 - what is line-listed?

Response: Thank you for the comments, in the updated version, traditional surgical operation practices were cited.
Line 55 data are always in plural?

Response: Thank you for the comments, it was a typo error and was corrected in updated version.

Is bivariate same as uni variate (examining one variate on the outcome?)

Response: Thank you for the comments, I think Univariate is like descriptive statistics where we summarize variables independently without assessing any relationship. In bivariate analysis, we examined one by one variable versus outcome, then in multivariable analysis all variables which where statistically associated with the outcome were considered in the model.

Not surprisingly age category is the main determinant for HCV exposure. Recommend you in the multivariate analyzes instead adjust for age using it as a continuous variable and not as an outcome category. It would probably give more insight into the other determinants Were the variables included in the final models examined for interactions?

Response: Thank you for the comments, before transforming age in categorical variables, we did analysis of it as continuous variables and saw that every year each participant has 0.1% of risk of developing HCV. So considered categorical variables for identifying the range of ages which need more interventions than others. Also, one-year increase is less meaningful than looking at specific categories. That is why we considered age as categorical variable. HBP and CRF were included in final model and we taught the association of both two variables with the outcome was influenced by age. Interactions between age and comorbidities were assessed and HBP and CRF were found to be interacted with age.

Without n’knowledge on the Rwanda health system one might imagine that private insurance would interact with high socioeconomic status and the same for Diabetes # high blood pressure#CRF and HIV # TB

Response: Thank you for the comments, more people in Rwanda are Mutuelle which is the only public health insurance. Private insurances are dominated by Insurance of civil servants and military insurance; less people are in other private insurances. We controlled insurance and wealth group in the model therefore, these factors shouldn’t influence the findings. For Diabetes, HBP and CRF are more prevalent in aged people and each socioeconomic class is concerned while HIV and TB are predominant in people between 25-45 years old. All of these variables were controlled in the model and they did not influence the findings.

It is stated that a number of variables were kept in if they were presumed intuitive or appropriate -but including number of sexpartners when 97.3 state the same answer (no more than 1) is very unlikely to add to the model
Response: Thank you for the comments. Because it was a self-reporting, we knew that we could face some bias that is why we reported this in limitations. For this manuscript version we did not consider them in model.

Results

Study population

Advice to be consistent with numbers - either 123456 or 123,456

Response: Thank you for the comments, it was a typo error and we used only 123456

Line 46-49 - Please proof read again could be more clear

Almost no participants (0.1%) were in the highest Socioeconomic category (Ubudehe 4) - is it then meaningful to have it as a separate category?

Response: Thank you for the comments, As Ubudehe category 4 had very few numbers of observations and was merged with category 3 in multivariable analysis.

It would be helpful with at short description on how Ubudehe categories are defined?

Response: Thank you for the comments, Ubudehe category was defined and more details were added in new version of the manuscript for more clarification. ‘Ubudehe is a development programme whereby citizens are placed into different socioeconomic categories. The lowest socioeconomic category is 1 and the highest is 4. Socioeconomic status was defined in accordance with the updated Ubudehe category by the Ministry of Local Government of Rwanda (MINALOC). The households that consume less than Rwandan Francs (RWF) 105,064 [equivalent to USD 120] per year are classified in Ubudehe Category 1 (poorest category), which includes people without houses and those affected by food insecurity. Households that consume between RWF 105,064 and RWF 159,375 (exclusively) [equivalent to USD 180] per year are in Ubudehe Category 2, which includes households subsisting on manual labor or temporary employment and those capable of renting or owning their own houses. Categories 1 and 2 receive government assistance. Households that consume more than or equal to RWF 159,375 are classified in Ubudehe Category 3 and 4.’

Try to minimize repeating figures stated in the tables in the text or try to rank them eg. "the main determinant for HCV seropositivity was xx with an aOr of XX and a p of xx indicating of major association

Response: Thank you for the comments, In the updated version we tried to do the summary by putting the main determinants of HCV seroprevalence in order to decrease repetition.
Line 32 - Being in Ubudehe category 3 had lower RAMA as…- is something missing here
Response: Thank you for the comments, this was corrected in new version.

Line 35 - mutuelle is a name? Capital first letter?
Response: Thank you for the comments, this was an error and Mutuelle is in capital letter.

Discussion

Includes limitations and comparison with other studies including the major limitation of age restricting the cohort and mixed effects or no causality proven by higher aOR for exposure among family members.

Response: Thank you for the comments, limitations and other studies were included in the updated manuscript.

The higher prevalence in the older age cohort is a historical exposure - but data cannot really prove this and a cumulative risk is just as likely?

Response: Thank you for the comments, you are right that we could include a cumulative risk but unfortunately due to cross sectional data we were unable to measure cumulative risk, for next studies with cohort data we will measure it.

It is hypothesised that migrants are the cause of higher prevalence outside the north region of Rwanda but being a migrant was not a factor in the data or were migrant excluded from the study?

Response: Thank you for the comments, this study was strictly done to Rwandan population and migrants were not considered in this study.

In the conclusion opportunities for prevention and screening is mentioned. Do the authors think screening should be universal, birth cohort or risk based on their finding?

Response: Thank you for the comments, we think it should be universal but do concentration effort on most at risk people shown in this study. ‘Screening should be universal and priority for testing and screening should be given to people who are at high risk such as older age, lower socioeconomic status, geographic variation, history of traditional surgical practices, and family exposures for both prevention and screening as Rwanda commits to achieving WHO targets of eliminating HCV by 2030.’
Line 8 - Please spell out PLHIV at first use

Response: Thank you for the comments, it was a typo error and was corrected.

Tables in general

Consider the headings - Frequency is used for number of participants(n) which is confusing

Response: Thank you for the comments, the frequency was changed to number of participants

There is on decimal in table 1, two in table 2 and 3 in table 3. Suggest one for percentages and max 2 for the OR and p value?

Response: Thank you for the comments, in updated manuscript we have 1 decimal for percentage and 2 for OR and p-value.