Author's response to reviews

Title: Prevalence and risk factors of hepatitis B and C virus infections among the general population and blood donors in Morocco

Authors:

Warda Baha (wardabaha@gmail.com)
Abderrahim Foulous (foulous@yahoo.fr)
Noureddine Dersi (ndersi@gmail.com)
Thierry Paluku They-they (thierrypal@yahoo.fr)
Khadija El alaoui (elalaouikhadija01@gmail.com)
Nadia Nourichafi (nadia_nourichafi@yahoo.fr)
Bouchra Oukkache (bouchraoukkache@hotmail.fr)
Fatiha Lazar (fatiha.lazar@pasteur.ma)
Soumaya Benjelloun (soumaya.benjelloun@pasteur.ma)
My Mustapha Ennaji (m.ennaji@yahoo.fr)
Abdelouhad Elmalki (abdelouhad.elmalki@pasteur.ma)
Hassan Mifdal (crtscasa@yahoo.fr)
Abdelouaheb BENNANI (abdelouaheb.bennani@pasteur.ma)

Version: 3 Date: 15 November 2012

Author’s response to reviews: see over
Dear Editor Board and reviewers,

First of all, we would like to thank you for agreeing to judge our work and for the relevant recommendations in order to improve the quality of the manuscript.

With the intention of adding information about the mapping of hepatitis B and C infections in North Africa and as most of the previous studies in Morocco were conducted in selected group of people with higher risk factors, the main objective of the present study was to report the prevalence of HBV and HCV infections among the general population and among blood donors from Casablanca, which is the most densely populated city in Morocco. Furthermore, we have investigated the risk factors associated with these blood borne infections, which is important to establish prevention strategies.

Regarding the reviewer’s comments, we’ve listed them to respond to each point:

**Reviewer: Louise-Anne McNutt**

This manuscript summarizes two seroprevalence studies conducted in Morocco, one in the general population and one in a blood donor population. The report is thoughtfully written and provides important information about hepatitis B and hepatitis C prevalence in the country. The researchers also provide basic risk factor information that is useful to understand the possible causes of HCV infection in the general population, in particular.

The following list provides minor comments that may be useful in revising the manuscript:

1. Overall the manuscript is well-written and easy to read. There are some copyediting issues that need to be addressed.

2. Conclusion for abstract: Alarming may be a somewhat overstatement of the HBsAg findings. Those under 20 have very low rates of HBsAg, consistent with an effective immunization program. The higher rate of HBsAg among adult men compared to women is noteworthy and suggests risk factor differences. Given that HBV is fairly easily transmitted sexually, this is interesting.
All the reviewers have agreed that the word "alarming" overstates the findings of this study, so we changed it and added some modifications to the abstract in regard to the suggestions.

3. Methods: “For population sampling, public and private bodies were randomly ...” is not clear. It looks like a multi-stage sampling method was utilized, however this needs to be clarified and a definition of “bodies” is needed.

Again, all the reviewers agreed that this part was not well detailed, so we tried to rewrite it to make it clearer to the readers.


- The prevalence odds ratio is estimating the prevalence ratio (not relative risk). This is because you don’t know which came first – exposure or infection. This is a simple wording change.
- Another wording change is that assessing the association of two factors is bivariate analysis, not univariate analysis.

Yes, we totally agree with you, we corrected the text.

- While odds ratios are mathematically acceptable because the outcome in not common, computing the prevalence ratios directly would be simpler for readers to interpret the results. This is not required because odds ratios are acceptable here, just a suggestion to consider.

Yes, you are right, thank you for the information.

5. Results (General Population)

- In the methods or discussion section it would be helpful to understand why the gender ratio is so far from 1. This may be solved by clarifying the sampling methods.

In the method section, we clarified the sampling method; samples were randomly selected by a systematic 1:3 sampling procedure from many establishments (public and private), which are also randomly selected. We think it is by chance; the number of men was higher than that of women, which obviously can reflect the sex ratio.

- Table 1 Except for age, the percentages look about right, however I would recommend computing the row percentages instead of the column percentages. Again,
there is nothing incorrect with computing column percentages. The reason for suggesting row percentages as you are discussion risk factors for HCV infection, thus row percentages communicate this information more clearly.

You are right; we agree with you and rectified the table by computing the row percentages.

➢ Age in Table 1 has a couple of issues. First, the percentages look like table percentages not column percentages (inconsistent with the rest of the table). Second, several age groups should be included as the data is interesting beyond a break-point at 40 years.

We rectified all percentages in the table 1 including that of age. We avoided putting the age groups in the table 1, as we have a null value in the first age group (none had HCV infection), we couldn’t estimate the confidence interval CI for this group.

6. Discussion:

➢ The first of “two main reasons” for HCV prevalence increasing with age is actually two different issues. One reason is related to screening blood for the virus. The second is nosocomial transmission due to non-sterile instruments, inappropriate reuse of supplies, etc. This second reason is the primary reason for HCV transmission in US and EU health care and many outbreaks have been documented.

Totally true, we corrected the idea in Paragraph 3 in discussion section: from “This can be supported by two major reasons… until the end of the paragraph”

➢ The second reason is more time at risk for exposure. This makes sense. However, some of the examples used are highly controversial. HCV is not easily transmitted through heterosexual sex or vertical transmission. More health care exposures is clearly a possible, and likely, reason for the age association. Other risk factors that may be too controversial to mention in the paper for Morocco are men who have sex with men and illegal injection drug use.

Certainly, the vertical transmission has not been shown to be among the main route of transmission of HCV, so we removed that idea from the text and kept the two main routes of transmission, namely, transmission through contaminated blood and iatrogenic transmission.
We have not introduced homosexual in our study because it is not common in our country as you mentioned, however among drug users there is another ongoing study at this time.

➢ Reference 11 appears to summarize an unusual study. I am not sure it is helpful for your paper as it may just be an anomaly. Rather, it may be that your reason for HBsAg decreasing after 50 makes sense and relates to infection at very young ages, while HCV is more likely contracted through health care at older ages. Selecting a more likely scenario as opposed to pointing out an unusual population may be more useful to your discussion.

In discussion section, paragraph 5, we changed this reference by another (3 and 5) of which the results were similar to ours.

**Reviewer: Manal H El-Sayed**

The study by Beha and co-authors is important as there are few studies reporting the prevalence of HBV and HCV in Morocco

There are some concerns and queries regarding the manuscript: Major Comments:

1. In the methodology section, the authors should highlight the sample size calculation in view of the previously known prevalence rates.

We did not mention the sample size in the methodology section, because the number of subjects recruited for HBsAg and anti-HCV detection was not the same. So we preferred to report it in the result section.

2. Eligibility criteria for recruitment are not clear for the general population. The authors should also state the numbers recruited from various centers and their distribution. Where patients consecutively or randomly recruited for the purpose of this study? Where questionnaires and training standardized for all participating centers?

This was nearly the same remark as that of Professor Louise-Anne (point 5). In the method section we clarified the sampling method. Patients were randomly recruited and the questionnaire is standardized for all participating establishments.

3. It is also not clear whether blood donors were consecutively enrolled.
As mentioned in method, blood donors section, subjects were volunteer blood donors who donated blood at the Casablanca Regional Blood Transfusion Center between January 1st, 2008 and December 31, 2010 and to be accepted to donate blood, they have to fulfill the selection criteria of the center. Therefore all people accepted were included in this study.

4. In the results section, the allocation of samples for studied tests should be clarified; 41269 samples were tested for anti-HCV and 23578 for HBsAg. The sample size is different and the authors don’t explain why some samples were tested for HCV and others for HBV. How many samples were tested for both HBV and HCV?

The sample size is different because at the beginning, the aim of the study was to investigate the prevalence of HCV only. When it was decided to study HBV, each subject recruited was enrolled for both HBsAg and anti-HCV detection. Thus 23578 is the number of samples tested for both HCV and HBV. It is mentioned in results section (general population) 6th paragraph.

5. The age range is quite diverse in the general population and risk factors should be identified by age groups.

We corrected the table I, which reports risk factors in the general population. As for the comment of Professor Louise-Anne (point 5.3), we avoided putting the age groups in the table 1, as we have a null value in the first age group (none had HCV infection), we couldn’t estimate the confidence interval CI for this group.

6. In the discussion section: The policies adopted by Morocco for blood screening should be elaborated. The authors are also advised to refer to previously published prevalence data (e.g Infectious risks associated with blood exposure for traditional barbers and their customers in Morocco by Zahraoui-Mehadji et al, 2004; Prevalence of hepatitis C virus infection in Morocco and serological tests assessment of detection for the viremia prediction by Benouda et al, 2009; Hepatitis B prevalence and risk factors in Morocco by Sbai et al, 2011; Epidemiologic and virologic study of hepatitis C virus infections in Morocco by Cacoub et al, 2011...)

To reduce the transfusion risk in Morocco, since 1994, all blood samples were routinely screened for transfusion-transmitted diseases (HBV, HCV, HIV and Syphilis). We mentioned that in method and discussion (paragraph 3) sections;
We added references from Morocco (ref 7, 8, 9 and 33).

Minor Comments

1. Typological, language and grammar mistakes should be reviewed and edited.

The manuscript was reviewed by English-speaking colleagues.

2. In the abstract, the studied population should be described. In the conclusion, alarmingly high prevalence doesn’t quite describe the findings of this study or previous WHO prevalence data which place Morocco in the intermediate prevalence zone.

Changes were made in the abstract regarding the suggestions.

3. The background section should be shortened and focused on local and regional data.

Changes were made in the background section and we added references of previous studies in the country.

4. The legend of the first table is incomplete and should state that the represented data belong to the general population.

We completed it.

5. In table 2, the percentage should have been calculated as mentioned in the upper horizontal axis.

We added it.

6. Some references are fairly old and should be updated.

We tried to change and add some references more recent (e.g, ref 7, 8, 9, 17 and 18).

Reviewer: Mohamed Abdel-Hamid

Minor Essential Revisions

➢ It is well written.

➢ This is a general survey of HBsAg and anti-HCV in general population and blood donors in Morocco.
I don't believe they can say the seroprevalence is "alarmingly high" since it is based upon older blood donors and is rather low.

All the reviewers have agreed that the word "alarm" overstates the findings of this study, so we changed it.

They have left out the percentages in table 2.

We rectified it.

The authors mentioned that there is a confirmation by RIBA in blood bank but they did not mention how the results were adjusted according to the RIBA results.

As mentioned in the last sentences in the 3rd paragraph of the method section (blood donors), Samples were considered to be positive if were reactive for both tests.

In Background:

Remove the last line of the 3rd paragraph starting with "These antibodies are present in ....." as it is irrelevant.

Rephrase the last 2 lines of the 4th paragraph

Add relevant reference to the last statement in the 4th paragraph (background)

All these comments were taken into consideration;

The 2 lines of the 4th paragraph “Although a few studies have been carried out, they are affected by a referral bias because, in most studies the population-based studies are limited (risk groups or blood donors) and information on young or elderly subjects, which is not included in such specialized groups, is lacking” were changed by “Although a few studies have been carried out, they were affected by a referral bias because most studies are generally based on subjects from risk groups or blood donors with lacking information on children and senior citizens not generally included in as specialized groups [7, 8, 9]”; 

We added the references (7,8,9).

In Methods:

Remove the word "among" after Morocco Remove the word "to" before "interview." Change "anti-VHC" to "anti-HCV" after "Murex." The kits used in the
assay in blood bank subjects should be specified. (They only mention a third and fourth generation ELISA.) The same for the PCR methodology.

The words were changed;

The references of kits were added in the method section;

For PCR it was mentioned in the last paragraph in method section (general population) that we used the Cobas Amplicor HCV Test (Roche diagnostics, Mannheim, Germany), (limit of detection 50 IU/ml).

In Results:

➢ It is better to change the word "processed" to "tested." You can remove "On the whole" before the seroprevalence. The paragraph starting with In terms of seropositivity needs to be rephrased in better English. The last paragraph in 2- blood donors you can remove the word "further."

All these comments were taken into consideration;

The paragraph 6 in the result section (general population) “In terms of the seropositivity of HBV and HCV dual infection, only two cases were detected in male subjects who belonged to age groups 20-29 years and ≥50 years respectively” was changed by “Regarding the seropositivity of HBV and HCV dual infection, the presence of both HBsAg and anti-HCV antibodies was noted in 2 cases Out of 23 578 tested patients. These two patients were male subjects who belonged to age groups 20-29 years and ≥50 years respectively”.

In Discussion:

➢ Change the word "infections" to"prevalence" in the 2nd line of first paragraph. Rephrase the last line of the first paragraph (HBV in the causation....) Change "measurable" to "detectable" viremia in the second paragraph. In the 3rd paragraph try to mention some specific areas from reference 12-13 instead of the regions in the world in general with adding specific figures. Change "in keeping with" to "similar to" in the last line of 3rd paragraph. Change HBIG to hepatitis B immunoglobulin (in the 4th paragraph).
All comments were taken into consideration.