Author's response to reviews

Title: Prevalence of Trichomonas Vaginalis Infection in Egyptian Women: Cross-Sectional Study

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Author's response to reviews: see over
Reviewer’s report

Title: Prevalence of Trichomonas Vaginalis Infection in Egyptian Women: Cross-Sectional Study

Version: 6

Date: 7 October 2014

Reviewer: Barbara Body

Reviewer’s report:

Major compulsory revisions:

1) Although the authors addressed why they chose not to look at other causes of vaginitis in their cover letter, they didn’t include this information in the manuscript. A simple statement such as, “We have previously studied other causes of vaginitis in our center and limited this study to a large scale cross-sectional study to assess the incidence of Trichomonas.” would be sufficient. Statement added at the beginning of the discussion (line 156, 157). I think this statement would be better added at line 65.: Statement added as recommended at the end of the aim of the study

2) The manuscript needs to clarify exactly what number of specimens were uniquely positive by culture, Giemsa, wet prep and Kalon. If Kalon detected all 50 positives – those positive by culture, Giemsa and Wet prep, that would be remarkable. As shown by table 1 (which was added in the previous revision): Culture detected 30 true positive, Kalon detected 50 positive (but 20 of them were false positive), Wet mount detected 10 Positives. The authors have missed my point. It is highly unusual that culture did not detect most of the specimens positive by Giemsa stain so the question is ‘Were there 30 specimen positive by culture and of those 30, ten were also detected by
The statements on lines 183-184 imply Giemsa and wet prep each detected 10 specimens not detected by culture. My experience this is not typical of the performance of the culture or smears.

<table>
<thead>
<tr>
<th>Test Used</th>
<th>M. Diamond Culture (Gold Standard Test)</th>
<th>Kappa value*</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>+ve (n= 30)</td>
<td>-ve (n= 970)</td>
<td></td>
</tr>
<tr>
<td>Kalon Latex</td>
<td>+ve 30 (100%) TP</td>
<td>20 (2.1%) FP</td>
<td>0.740</td>
</tr>
<tr>
<td></td>
<td>-ve 0 (0%) FN</td>
<td>950 (97.9%) TN</td>
<td></td>
</tr>
<tr>
<td>Giemsa Stain</td>
<td>+ve 10 (33.3%) TP</td>
<td>0 (0%) FP</td>
<td>0.492</td>
</tr>
<tr>
<td></td>
<td>-ve 20 (66.7%) FN</td>
<td>970 (100%) TN</td>
<td></td>
</tr>
<tr>
<td>Wet Mount</td>
<td>+ve 10 (33.3%) TP</td>
<td>0 (0%) FP</td>
<td>0.492</td>
</tr>
<tr>
<td></td>
<td>-ve 20 (66.7%) FN</td>
<td>970 (100%) TN</td>
<td></td>
</tr>
</tbody>
</table>

* a measure of agreement between the test and modified Diamond culture

**TP:** TRUE POSITIVE  
**FP:** FALSE POSITIVE  
**TN:** TRUE NEGATIVE  
**FN:** FALSE NEGATIVE

3) I don’t find credible the high percentage of specimens positive uniquely by Geimsa and wet prep (& possibly Kalon), respectively compared to culture. This is not comparable to data in the literature which would typically show wet prep or Geimsa to detect about 50% of those specimens positive by culture. This unusual performance needs explanation. Wet mount and Giemsa detected ONLY 10 positive cases – were those the same 10 in each case and were they also detected by culture?, Kalon detected 30 positive cases out of the whole 50 positive cases (as the other 20 positive cases detected by Kalon were false positive when compared to Culture), and this is comparable to Literature (Table 1).

Modified Diamond culture was the gold standard test used that detected 30 positive cases for trichomonal infection out of the 1000 women studied. Kalon LATEX agglutination detected 50
cases as positive where 20/50 cases would be considered false positive considering the gold standard test for comparison. To confirm whether the false positives are truly so, more sophisticated tests such as PCR would be needed that are superior to culture, as you have kindly mentioned earlier in your first comments. This could be the scope of future studies.

Out of the 30 positive cases detected by culture as gold standard, 10 of which were also positive both on Giemsa staining (10/30 i.e 33.3%) and on wet mount using light microscopy (10/30) i.e the same 10 were positive for Giemsa and wet mount. In other words, both Giemsa stain and wet mount could only detect 33.3% of those specimens that were positive by culture Thus, detection by Giemsa stain and wet mount accounted only for 1% of cases positive for Trichomonal infection. This agrees with the literature where culture is a superior method for detection, however more tedious and time consuming.

All 30 cases positive for Trichomonal infection by culture were also positive upon Kalon latex agglutination. In addition, Kalon test detected 20 more cases as positive which were not detected by culture leading to a 100% sensitivity, however a PPV of only 60%.

In other words, as we have mentioned in our discussion, a positive Kalon test is in itself poor at confirming Trichomonal infection (PPV = 60%) and further investigations must be undertaken; it did, however, correctly identify 100% of all cases (the sensitivity). However as a screening test, a negative result is very good at reassuring that a patient does not have the infection (NPV =100% ) and at this initial screen correctly identifies 97.9% of those who do not have trichomonas infection (the specificity)
4) The focus of this manuscript and therefore the conclusions and recommendations should be statements pertaining to the testing of women for Trichomonas vaginalis. It is not appropriate for the conclusion & recommendation section to make conclusions about the reliability of the Kalon latex agglutination test reliability. This manuscript has not presented data that demonstrates by discrepant analysis that the results of the Kalon test were correct. The conclusion and recommendations focused on Latex agglutination, coming from its availability, easiness, relatively cheap (in our community), and not time consuming, in order to consider adding it as a screening test in the gynecology clinics in Egypt (at least university hospitals) (as done with Pap smear for cancer cervix...) as it will help in rapid and more or less accurate screening, and start treatment for cases suspected to have T.vaginalis infection.

We aimed to study the prevalence of trichomonal infection per se, not knowing what to expect. We retrospectively found that the prevalence of infection was relatively low inspite of the symptomatizing women which could have been possibly suffering from other forms of vaginitis but with a similar clinical picture.

Thus, the overall prevalence of Trichomonas infection using all methods of detection collectively (Giemsa staining, wet mount, culture and Kalon agglutination test) was 5% among the studied population of women which could be an overestimated prevalence until superior and sophisticated tests as PCR are performed, to confirm or refute the 20 more cases that were Kalon test positive, however culture negative. However, a lower prevalence of 3% was obtained using modified Diamond’s culture alone as a gold standard test for detection being superior to all methods used in this study.
The low prevalence can be possibly attributed to the social and cultural conservative nature of the Egyptian society regarding free sexual relations. Other causes of vaginitis such as candidiasis or bacterial vaginosis have been extensively studied at our centre with no emphasis or large scale study on trichomoniasis prevalence.

We can possibly modify the title as: **Prevalence of Trichomonas Vaginalis infection among Egyptian women using culture and Latex agglutination: a cross-sectional study**

This is in fact a cross-sectional study to detect the prevalence of Trichomonal infection where we used various methods for diagnosis namely, the modified Diamond culture as the gold standard test, the Kalon Latex test, wet mount with light microscopy and Giemsa stain to improve the detection rate while comparing the accuracy of these tests in detection.

Moreover, a paragraph was added in the conclusions to clarify our points (line 197- 204)

5) Photos are unlabelled and without narrative duplicate photos are still present and need to be removed for the Giemsa stain and wet prep. For the Wet Prep photo, I would use only the small photo on the upper left. Label to the photos added. Two photos of Giemsa are still present – one needs to be deleted. Duplicate photo removed, and narration added to all the 3 figures

**Minor essential revisions**
There are still numerous typographical errors: revised
Level of interest: An article whose findings are important to those with closely related research interests

Quality of written English: Acceptable

Statistical review: Yes, and I have assessed the statistics in my report.

Declaration of competing interests:
I have participated in clinical trials for the FDA clearance of nucleic acid amplification tests from two manufacturers. I did not receive compensation but the company for which I work was paid to perform the testing. I do not have any other financial or non-financial competing interests