June 23, 2025

To: Editors, *Viruses*

Re: Revisions to Manuscript viruses-3649947 entitled " *Trichomonas vaginalis* Virus: Current Insights and Emerging Perspectives"

Dear Journal Editor(s),

Thank you for the review of our manuscript and the request for revisions. We have responded to each of the reviewers' comments as listed below and are submitting a revised manuscript (compare copy) with tracked changes in red and a clean version. Please do not hesitate to contact us should you have any questions about our revisions or request additional revisions.

Reviewer(s)' Comments to Author:

Reviewer #1 (Comments for the Author):

In this manuscript, the authors review previous studies on *Trichomonas vaginalis* virus (TVV), including its virology and potential effects on the parasite host. However, most of the points discussed have already been reported in the literature. For example:

1. *Trichomonasvirus: a new genus of protozoan viruses in the family Totiviridae* [DOI: 10.1007/s00705-010-0832-8]
2. *Trichomonas vaginalis virus: a review of the literature* [DOI: 10.1177/0956462418809767]

Notably, the second reference was published by the same authors as this manuscript, and there appears to be little new information compared to their previous work. Several specific concerns are outlined below:

1. **Lines 18 & 128–137:** The manuscript states that TVV can be transmitted via cell division or through small extracellular vesicles. However, this has not been experimentally confirmed. The authors should revise this section to clarify that the transmission mechanisms of TVV remain uncertain, and that vertical transmission is only a proposed hypothesis based on limited evidence.

2. **Lines 18–25 and Section 3 (starting at Line 138):** The authors suggest that TVV influences virulence, drug resistance, and protein expression in *T. vaginalis*. However, several studies have reported no such associations. To provide a more balanced view, the authors should acknowledge these contradictory findings and offer a discussion on possible reasons for the discrepancies.

3. **Line 109:** The text implies that satellite RNAs are part of the viral genome. However, in the cited reference, the satellite RNAs were found in virus-positive isolates but are not components of the viral dsRNA genome. The authors should revise this statement to clarify that these RNAs may act as auxiliary elements necessary for replication, rather than being part of the viral genome.

4. **Line 172:** The manuscript refers to vesicles larger than 200 nm as "large vesicles," but the correct term is "microvesicles." Furthermore, the cited reference does not define or mention microvesicles. The terminology should be corrected for accuracy, and the appropriateness of the citation should be re-evaluated.

Author Response: Thank you to the reviewer for these comments. Below are the changes made to address these suggestions.

We were invited by the *Viruses* editors to write this review on TVV to commemorate the 15-year anniversary of the journal. While we acknowledge the 2011 (PMID: 20976609) and 2019 (reference #23, PMID: 30626281) review papers, we have attempted to include new research on TVV that has been published since these prior papers (i.e. identification of the 5th TVV in 2023 (reference #27, PMID: 35336955) as well as the possible use of *T. vaginalis* microvesicles as a viral transmission mechanism(reference #61, PMID: 35602021)).

1. We agree with the reviewer on this point. We have added language to better reflect that these two transmission mechanisms are hypothetical and based on the limited evidence reported from the respective studies. These corrections are in lines 18 and 130-134.
2. We agree with the reviewer. We have balanced these sections by including statements acknowledging the contradictory findings. The additions can be found in section 3 [lines 147-149].
3. We agree with the reviewer's comment. We have corrected this section of the text to more accurately reflect the relationship between the viral dsRNA and the parasites satellite RNAs. These corrections are in lines 110-112.
4. We have corrected the terminology to the use of "microvesicles" and included the appropriate citations. These changes can be found in lines 175-181.

Reviewer #2 (Comments for the Author):

This review provides an excellent summary of the current knowledge regarding TVVs and their role in the pathogenesis of *Trichomonas vaginalis*.

There are few minor comments related with the manuscript which I list below:

1. Fig 1 is slightly blurry.
2. Line 114: The dot after "Manny et al" is missing
3. Lines 140, 141,147,151,153 and 154: T. vaginalis is not in italics
4. Line 161: please change the word "strain" to "isolate"
5. Line 186: the sentence can be a bit confusing for the reader. I recommend simplifying it by using an expression such as 'TVV+ isolates' or 'parasites/trichomonads harboring TVV'.
6. Line 207: specify that those interferons and cytokines are proinflammatory
7. Regarding the paragraph related with symptoms and TVV, only Fraga et al., 2012 have reported that association . I recommend to highlight this aspect.

Author Response: Thank you to the reviewer for these comments. Below are the changes made to address these suggestions.

1. A higher resolution version of Figure 1 has been added to the manuscript.
2. The correct punctuation has been added to the "Manny et al." citation in line 116.
3. *"T. vaginalis"* has been italicized where mentioned.
4. We agree with the reviewer on this point. The word "strain" has been replaced by "isolates" to better reflect what we intended in line 166.
5. The uses of "TVV-positive/negative clones of *T. vaginalis*" in lines 187-191 have been replaced with the use of "TVV-positive/negative *T. vaginalis* isolates". We believe this will remedy any confusion experienced by readers.

6. We have specified that the interferons and cytokines are proinflammatory in line 212.
7. There is a second article that also details associations between patient symptoms and TVV presence cited in El-Gayar et al. 2016. We have added language that highlights this aspect [lines 213-214].

Once again, please do not hesitate to contact us should you have any questions about these revisions or require any additional information.

Sincerely,



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