Response to Reviewer 1 Comments

In this manuscript the authors (Kononova et al.) describe their investigations of the ability of a number of fish and reptile derived cell lines to support the replication of SARS-CoV-2, the rational being that species within those animal groups might get exposed to the virus via waste water, sewage, flood run-offs, contact with birds and other species, etc., notably around major cities and from intensive farming enterprises. Employing five such cell lines (three fish-derived, two reptile-derived), they test the cells at different temperatures and with different virus challenge doses (the MOI should be provided rather than or in addition to the inoculum size). Vero cells are used as the comparator, tested at both 34C and the optimal temperature of 37C. As read out, they assess the cells for CPE, as well as conduct RT-PCR for viral RNA in tissue culture supernatant and virus titration on Vero cells. Only a turtle-derived cell line (TH-1), grown at 29C, supports SARS-CoV-2 replication to a level comparable to Vero cells at 37C, with the virus inducing extensive CPE in the cells. Viral replication does also occur to a limited degree in a crocodile-derived cell line, but CPE is not seen. No virus replication is detected in the three fish cell lines.

While these studies do not prove that fish are resistant to SARS-CoV-2, the results may indicate that it would only be in special circumstances that infection would take place and one might question whether it would be sustainable. In contrast, reptilians might – at least under high ambient temperatures – be able to support virus replication, but it remains to be ascertained whether this would happen to a degree, where they could sustain a reservoir in nature such as has been see with white-tailed deer in North-America (PMID: 37640694). Crocodilians, in particular, are known to be able to contribute to a transmission cycle of some human pathogenic viruses, notably arboviruses (e.g., PMID: 35114499, 35632847, 32054016). With climate change and ever-increasing environmental temperatures this could become of some concern.

The Discussion is very long with a lot of reiteration of results, and should be shortened to focus on the main points derived from the study. Also, Figure A1 could be incorporated into the manuscript as Figure 3. Figures 1 and 2 will need improvement to enhance sharpness/focus. Currently they are quite blurry.

We thank the Reviewer 1 for comments and suggestions aimed to improve our result presentation. In the manuscript we highlighted any revision using the green highlight function. A few minor corrections that were made to the text have not been listed in this letter but were highlighted in the revised document. To improve result presentation, we also edited Figures.

Point 1. Line 23: delete ‘that’

Response 1. Thank you. It was corrected.

Point 2. Lines 38-40: this sentence needs rephrasing for improved syntax and greater clarity.

Response 2. We appreciate your recommendation. The sentence was changed.

Point 3. Line 44 & throughout the manuscript: Latin species names should be written in Italics.

Response 3. All names were checked and corrected. Thank you.

Point 4. Line 55: correct to “and in nature”

Response 4. Corrected, thank you.

Point 5. Line 58: change to “documented in 26 mammalian species ……”

Response 5. Corrected according to your recommendations.

Point 6. Line 71: change to "from feces and urine are uncommon” (or infrequent).

Response 6. We changed the sentence. Thank you.

Point 7. Line 75: change to “and river waters is likely due to low …..”

Response 7. The sentence was rephrased.

Point 8. Line 91: suggest change to “are likely underestimated”.

Response 8. Thank you. Corrected according to your comment.

Point 9. Lines 116-118: this sentence is unclear and should be rephrased.

Response 9. We tried to make the sentence clearer. Thank you.

Point 10. Line 41: reference no. 46 is apparently in Russian and the methodology should therefore be described in details here, as it is unlikely that the majority of readers of VIRUSES understand Russian.

Response 10. We appreciate your recommendation. The reference was changed to the other one with method description.

Zhukhovitsky V, Shevlyagina N, Zubasheva M, Russu L, Gushchin V, Meerovich G, Strakhovskaya M. Infectivity and Morphology of Bovine Coronavirus Inactivated In Vitro by Cationic Photosensitizers. Viruses. 2022 May 15; 14(5):1053. doi: 10.3390/v14051053.

Point 11. Line 142: change to “All experiments were carried out …….”

Response 11. Corrected, thank you.

Point 12. Line 147: change to ‘experiments’

Response 12. We changed the sentence.

Point 13. Table 1: change ‘4 passage’, ‘7 passage’ and ’22 passage’ to either “4th passage” or “passage 4” etc.

Response 13. The Table was corrected according to your suggestions, thanks.

Point 14. Line 160: since the term ‘fetal bovine serum’ and the abbreviation FBS is used later and in Table 1, then it would be appropriate to also use “fetal bovine serum” here and introduce the abbreviation FBS here rather than in line 165.

Response 14. We made a transcript in the table, then abbreviated it. Thank you.

Point 15. Line 165 and elsewhere in the manuscript: TCID50 should be written with the 50 as subscript.

Response 15. Corrected as recommended.

Point 16. Line 256: please reorganize the order of the parentheses. Also, it is unclear why the genus names are repeated in the parenthesis.

Response 16. We appreciate your recommendation. All genera of the family Coronaviridae we corrected to italics. We suppose that Integrated spelling of the family is allowed.

Point 17. Lines 277-8: change to “with human and animal alpha- and beta-coronaviruses

Response 17. Thank you. Corrected as recommended.

Point 18. Line 288: delete ‘coronavirus’ (the word is already included in the abbreviation).

Response 18. It was deleted. Thank you.

Point 19. Line 304: delete ‘virus’ (the word is already included in the abbreviation).

Response 19. It was deleted. Thank you.

Point 20. Line 315-6: correct to “sensitivity to various viruses of warm-blooded animals”

Response 20. Corrected as recommended.

Point 21. Line 339: something seems to be missing between ‘observed’ and ‘SARS’?

Response 20. Thank you for the comment. We deleted ‘SARS’.

Point 22. Line 356: delete ‘was’

Response 22. Thank you for the comment. We deleted ‘was’.

Point 23. Lines 378-84: this is a lot of repetition of details from the Results section and should be cut out or shortened.

Response 23. We appreciate your recommendation and shortened details. Thank you.

Point 24. Line 413: correct to “infection, TH-1 cells incubated at 29C were comparable ……”

Response 24. Corrected as recommended.