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PEER-REVIEW REPORT

Name of journal: World Journal of Virology
Manuscript NO: 36851
Title: Identification of various cell culture models for the study of Zika virus
Reviewer's code: 03387199
Reviewer's country: Brazil
Science editor: Fang-Fang Ji
Date sent for review: 2017-10-27
Date reviewed: 2017-10-27
Review time: 14 Hours

CLASSIFICATION	LANGUAGE EVALUATION	SCIENTIFIC MISCONDUCT	CONCLUSION
[ ] Grade A: Excellent	[Y] Grade A: Priority publishing	Google Search:	[ ] Accept
[ ] Grade B: Very good	[ ] Grade B: Minor language polishing	[ ] The same title	[ ] High priority for publication
[Y] Grade C: Good	[ ] Grade C: A great deal of language polishing	[ ] Duplicate publication	[Y] Rejection
[ ] Grade D: Fair	[ ] Grade D: Rejected	[ ] Plagiarism	[ ] Minor revision
[ ] Grade E: Poor		[Y] No	[ ] Major revision
		BPG Search:	
		[ ] The same title	
		[ ] Duplicate publication	
		[ ] Plagiarism	
		[Y] No	

COMMENTS TO AUTHORS

Himmelsbach's work was very well designed and executed. Most of the experiments were well executed and the results are consistent with the conclusions of the paper. However, it should be noted that most of the presented results had already been described in other works separately. Furthermore, the importance/utility of demonstrating the ability of the virus to replicate in different cell lines (except for neural lines) was unclear. Vero and C6/36 cells have been used with great success in the multiplication of this virus. Therefore, this work brings very few advances in scientific knowledge. Major reviews: 1. The origin of the cells used should be described more clearly. Another important point is to present the passage used in the experiments since this fact can greatly influence the results of the experiments. 2. Why were two different methodologies used to evaluate intracellular and extracellular viral RNA? 3. The experiment to analyze changes in the Interferon pathway should be reviewed. It is

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known that DNA transfection into cells can alter various intracellular conditions, and infection immediately after transfection may have been compromised. Luciferase values should be normalized with an uninfected (mock-infected) control. 4. In the results it was described that the western blot experiment of NS1 expression was higher or lower in the cell lines tested, however, it has not been described in the methods what methodology was used to quantify the expression of said protein. 5. I believe that the LDH methodology should be better described, since it is not such a commonly used method. 6. In the fifth line of the discussion it was described that the Polynesian isolate is genetically close to that of Brazil. In fact he is the closest, compared to the African, but it is not so close. Minor reviews: 1. In the seventh line of the last paragraph of the introduction there is a punctuation error in the sentence. 2. In the third line of the topic "Cell culture", the correct spelling is L-Glutamine 3. The section describing the viral isolate used in the study should be placed in a more initial part of the material and methods. Also, add in which viral passage the isolate was used - this characteristic may influence the results. 4. Last line of results separate the “293T” from “cells”.

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PEER-REVIEW REPORT

Name of journal: World Journal of Virology
Manuscript NO: 36851
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Review time: 7 Days

CLASSIFICATION	LANGUAGE EVALUATION	SCIENTIFIC MISCONDUCT	CONCLUSION
[ ] Grade A: Excellent	[Y] Grade A: Priority publishing	Google Search:	[Y] Accept
[Y] Grade B: Very good	[ ] Grade B: Minor language polishing	[ ] The same title	[ ] High priority for publication
[ ] Grade C: Good	[ ] Grade C: A great deal of language polishing	[ ] Duplicate publication	[ ] Rejection
[ ] Grade D: Fair	[ ] Grade D: Rejected	[ ] Plagiarism	[ ] Minor revision
[ ] Grade E: Poor		[Y] No	[ ] Major revision
		BPG Search:	
		[ ] The same title	
		[ ] Duplicate publication	
		[ ] Plagiarism	
		[Y] No	

COMMENTS TO AUTHORS

The manuscript is well written providing information on susceptibility of different cell linex to ZIKV infection. This information is of importance for those working in the field and provides a basis for future studies. Introduction is to the point, results are clearly presented, and conclusions are supported by obtained data.

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PEER-REVIEW REPORT

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CLASSIFICATION	LANGUAGE EVALUATION	SCIENTIFIC MISCONDUCT	CONCLUSION
[ ] Grade A: Excellent	[ ] Grade A: Priority publishing	Google Search:	[ ] Accept
[ ] Grade B: Very good	[ ] Grade B: Minor language polishing	[ ] The same title	[ ] High priority for publication
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		[ ] Duplicate publication	
		[ ] Plagiarism	
		[Y] No	

COMMENTS TO AUTHORS

The manuscript by Himmelsbach et al. investigates a range of common established mammalian cell lines for their ability to propagate Zika virus. The final outcome provides an incremental advancement in the field and the study could be strengthened by addressing a number of issues listed below. Major comments: 1. Clarification on why ZIKV strain French Polynesia was chosen for the study should be provided. Is the most relevant with respect to disease incidence? Is this a lab adapted strain or a true low passage clinical strain? How divergent are the strains of ZIKV? If divergent it would be worth testing multiple strains. 2. No NS1 is apparent in figure 1c for ZIKV-infected SHY5Y cells in contrast to the statement in the results that lower amounts of NS1 were detected for SH-SY5Y? This needs to be clarified. It would be helpful if the band intensities of NS1 were quantified and normalised to the loading control actin to provide a quantitative figure for comparison with figure 1b. 3. Given CHO cells do not support

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ZIKV infection apparently at the stage of entry and the CHO cells were viable it would be extremely interesting to know if they lack the receptor for ZIKV entry. If the receptor is known then an experiment showing lack of surface expression in CHO should be included. 4. For figure 1d quantification of infected vs non-infected cells should be performed and both raw cell counts and ratios included in a table. 5. For figure 4a the differences in transfection efficiency between cell lines needs to be accounted for by normalising to a transfected plasmid expressing GFP (can be quantified by flow cytometry for example). 6. Figure 4b requires an uninfected control for comparison (results text actually refers to the uninfected control) and inclusion of the N29.1 cell line which based on figure 4a has an opposite interferon response compared to A549. 7. In the discussion the statement that the findings with 293T cells is in contrast to a published report needs further elucidation? Was the infection levels the same. Can the authors be sure of the integrity of their 293T cell line. STR profiling, where possible, of their cell lines would be highly beneficial. 8. The last two sentences in the 4th paragraph of the discussion make no sense? 9. Further explanation on why CHO cells would have reduced interferon response if not infected need to be provided. This could be due to differences in transfection efficiency rather than cell line differences. See point 5 above. Minor comments: (1) Introduction 2nd paragraph: a reference(s) is required for Zika virus classification and properties. (2) Materials and methods section on "ZIKV strain" should be immediately after "Cell culture" and "Virus titration assay" should come immediately after "Infection procedure". (3) Information on which region of the Zika virus genome the primers for qPCR actually map to should be provided. (4) RPL is not defined? (5) In materials and methods under virus titration assay should state "serial dilution of either cell culture supernatant or cell lysates….." (6) Need to clarify that the Flavivirus group antigen antibody is the same as in used in figure 1d and 4b which is described as virus envelope specific? (7) In materials and methods under "transfection and … assay" need to define components of the "passive lysis buffer"? (8) For figure 1c no mention of westerns or the antibody against NS1 is made in the materials and methods? (9) Scale bars should be shown in figure 1d and 4b. (10) Figure 4a legend presumably the authors mean uninfected Vero cells as reference? (11) For Table 1 a more appropriate heading than "inventor" would be "origin" or "source"

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PEER-REVIEW REPORT

Name of journal: World Journal of Virology
Manuscript NO: 36851
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CLASSIFICATION	LANGUAGE EVALUATION	SCIENTIFIC MISCONDUCT	CONCLUSION
[ ] Grade A: Excellent	[ ] Grade A: Priority publishing	Google Search:	[ ] Accept
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		[ ] Duplicate publication	
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		[Y] No	

COMMENTS TO AUTHORS

Kiyoshi Himmelsbach and Eberhard Hildt reported an investigation on ability of Zika virus replication using a total of 10 human and non-human cell lines to identify cell culture models for Zika virus. The experiments were conducted by infecting cell lines and analyzing 48h post infection intracellular and extracellular viral genomes and infectious viral particles using qPCR and plaque assay. Additional immunofluorescences and western blot analysis for Env and NS1 antigen were also used. Finally, authors analyzed interferon response into cellular context as innate immunity response. Overall it was reported that except CHO cells all cell lines supported Zika infection showing different cytopathic effect (CPE) in different cells (high CPE for A549 and Vero cells). Moreover different genomic yield versus infectious particles was reported at least in supernatant. No strict correlation between viral particles and interferon response was observed. The issue is of interest and the study was conducted appropriately to obtaine

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the goal of the investigation. However, there are aspects in method thad should be clearify. Main Points 1- The authors produced Zika virus in Vero cells and use it for the infection experiments of different cell lines. In doing so, the virus replication in Vero cells would be better than that obtained in other cells for its adaptation to growth in this cells. Why the virus was not produced in each specific cell lines and used directly in the experiments ? Can the author add some comments ? Moreover, the time of virus inoculum cell-incubation in the cell lines was 16 h. Why so long time ? For example in the titration assay it was used a 2 h time of incubation. The author should explain or comment this differences. 2- In the paragraph "Analysis of the amount and subcellular distribution of ZIKV envelope protein by confocal immunofluorescence microscopy it is not clear as the author obtained the percent of susceptibility. How has it been calculated? 3- Few cell could have a deficient interferon response. How this the authors can comment in their investigation ? Minor points 1- Introduction, line 4: the authors should explain the name of vector specificity (mosquito ?). 2- Introduction, line 18: Add the word "virus" after Spondweni. 3- Introduction, lines 20-25: Add references. 4- Materials and methods. In RNA isolation and cDNA synthesis it should be explained the amount of RNA used for retrotranscription. 5- Materials and methods. It should be explained if the qPCR used for the supernatant is an One-step qPCR or not. 6- Materials and methods. The ZIKV strain paragraph should be move after the cell culture paragraph. 7- Figure 1: the difference showed in figure 1a are not to be consider in the reason of that the log difference obtained can be a mere fluctuation of the quantification. 8- Results, line 16: "about 10^5-fold" should be changed in "up to 10^5-fold".