Dear editor,

Thank you very much for giving us this opportunity to revise our manuscript and resubmit it. This is a resubmission of the manuscript entitled “ Neonatal rhesus monkeys as animal model for rotavirus infection ” with a manuscript NO 41562.

We thank the reviewers for their careful reading and thoughtful comments on our manuscript. Those comments are all valuable and very helpful for revising and improving our article. We have carefully taken their comments and responsed point-to-point to the reviewers’s comments. If you have any question about this paper, please don’t hesitate to let me know.

Yours sincerely,

Na Yin

Reviewer1# : Correction required in Line 653 Page 26 Manuscript 41562 Line 653 Page 26/27 Table S1 heading Gastrogavage rout : please correct as route.

Respose: Thank you for your suggestions. We have revised this word to “route” in Table S1.

Reviewer2# : AUTHORS This is an interesting manuscript. The aims targeted by authors do value important results and I consider that authors have used an interesting methodological approach. The use of English language and the references used throughout the text are robust. I do have a few questions Authors say they have performed a neutralizing antibody test to confirm that the monkeys did not have antibodies against RV SA11 prior to the study. Please provide detail on how this test was performed (in-house or commercial, etc) Authors say that viral RNA was isolated from fresh tissue of experimental animals by Trizol and from venous blood by QIAamp viral RNA mini kit. Please provide details if extraction has occurred according to the manufacturers instructions. Please delete reverse primer information (I believe it duplicated) Did author check for viral inhibition in sample extraction and amplification? Would a false negative qRT-PCR result provide lack of sensitivity and misinterpretation? Please comment

(1) I do have a few questions Authors say they have performed a neutralizing antibody test to confirm that the monkeys did not have antibodies against RV SA11 prior to the study. Please provide detail on how this test was performed (in-house or commercial, etc).

Respose: Thank you for your comments. We provided details on how neutralization test was performed in the materials and methods section of the manuscript.

Neutralization test

The activated RVs were adjusted 1000 PFU/100 μL in serum-free MEM. The serum samples were diluted from 1:10 to 1:1280 in 100 μL serum-free MEM. The diluted RVs and serum samples were mixed with each and incubated at 37 ℃ for 1 h. The mixtures were transferred to the 96-well plates covered with a confluent monolayer of MA104 cell and were cultured at 37 ℃ for 5 days. The cultures were completely transferred to the wells of the ELISA plate coated with a goat anti-RV polyclonal antibody (Millipore, AB1129) and blocked with 3% (w/v) BSA (Biosharp, BS043D). The cultures were inoculated at 37 ℃ for 1 h. A rabbit anti-RV polyclonal antibody (prepared by the Department of Molecular Biology, Institute of Medical Biology, Chinese Academy of Medical Science & Peking Union Medical College) conjugated with horseradish peroxidase (HRP) was used to detect RV antigen at dilution of 1:2000 (v/v) in PBS at 37 ℃ for 1 h. All ELISA were developed using TMB (TIANGEN, PA107-01) to generate a colorimetric reaction and terminated by 2 mol/L H_2SO_4. The absorbance was read on a universal microplate reader (Elx800, Bio-Tek, USA) at 450 nm and 630 nm. A serum specimen was determined to be positive if the OD value was less

than or equal to the average OD value of the negative control ×2. The neutralization titers were defined as the highest dilution.

(2) **Authors say that viral RNA was isolated from fresh tissue of experimental animals by Trizol and from venous blood by QIAamp viral RNA mini kit. Please provide details if extraction has occurred according to the manufacturers instructions.**

Respose: Thank you for your comments. We provided details on how the extraction of virus RNA was performed in the materials and methods section of the manuscript.

Viral RNA Extraction

Viral RNA was isolated from fresh tissue of experimental animals by Trizol (Ambion, 15596026) . 1mL Trizol was added to 50mg~100mg of fresh tissue and the sample was grinded on the ice with an electric grinder. The homogenized sample was incubated for 5~10 minutes at room temperature. 0.2 mL of chloroform was added to the homogenized sample. The tube was shaked vigorously by hands for 15 seconds and incubated for 15 minutes at room temperature. Next the tube was centrifuged at 12000rpm for 15 minutes at 4 ℃ and the aqueous phase was transfered to a clean tube. 0.5 mL isopropyl alcohol was added to the tubes. Then the tube was incubated for 10 minutes at room temperature and centrifuged at 12000rpm for 10 minutes at 4 ℃. The supernatant was removed and the

RNA was washed with 1mL 75% ethanol and the tubes were centrifuged at 12000rpm for 5 minutes at 4 ℃. Then RNA were washed again and dryed for 5~10 minutes at room temperature. Finally, RNA were completely dissolved in 30μL RNase-free water and stored at -70 ℃.

At the same time, viral RNA was isolated from venous blood samples using the QIAamp Viral RNA Mini Kit , according to the manufacture's protocol (Qiagen, 52904)： Pipet 560 µl prepared Buffer AVL containing carrier RNA into a 1.5 ml microcentrifuge. Add 140 µl serum to the Buffer AVL–carrier RNA in the microcentrifuge tube. Mix by pulse-vortexing for 15 s. Incubate at room temperature (15–25 ℃) for 10 min. Briefly centrifuge the tube to remove drops from the inside of the lid. Add 560 µl ethanol (96–100%) to the sample, and mix by pulse-vortexing for 15 s. After mixing, briefly centrifuge the tube to remove drops from inside the lid. Carefully apply 630 µl of the solution from step 5 to the QIAamp Mini column (in a 2 ml collection tube) without wetting the rim. Close the cap, and centrifuge at 6000 x g (8000 rpm) for 1 min. Place the QIAamp Mini column into a clean 2 ml collection tube, and discard the tube containing the filtrate. Carefully open the QIAamp Mini column, and repeat step 6. If the sample volume was greater than 140 µl, repeat this step until all of the lysate has been loaded onto the spin column. Carefully open the QIAamp Mini column, and add 500 µl Buffer AW1. Close the cap, and centrifuge at 6000 x g (8000 rpm) for 1 min. Place the QIAamp

Mini column in a clean 2 ml collection tube (provided), and discard the tube containing the filtrate. Carefully open the QIAamp Mini column, and add 500 µl Buffer AW2. Close the cap and centrifuge at full speed (20,000 x g; 14,000 rpm) for 3 min. Place the QIAamp Mini column in a new 2 ml collection tube (not provided), and discard the old collection tube with the filtrate. Centrifuge at full speed for 1 min. Place the QIAamp Mini column in a clean 1.5 ml microcentrifuge tube (not provided). Discard the old collection tube containing the filtrate. Carefully open the QIAamp Mini column and add 60 µl Buffer AVE equilibrated to room temperature. Close the cap, and incubate at room temperature for 1 min. Centrifuge at 6000 x g (8000 rpm) for 1 min.RNA was stored at -70 ℃.

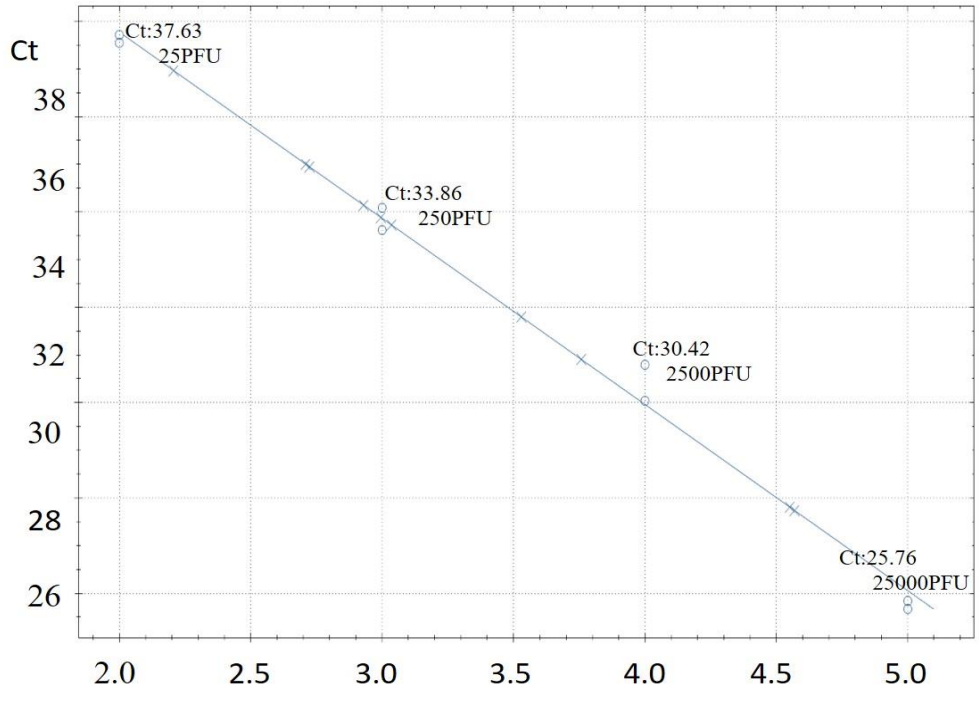
(3) Please delete reverse primer information (I believe it duplicated).

Respose: Thank you for your comments. Due to our carelessness, we wrote a repetition. We deleted it.

(4) Did author check for viral inhibition in sample extraction and amplification? Would a false negative qRT-PCR result provide lack of sensitivity and misinterpretation?

Respose: Thank you for your comments. In this study, we detected viral load variations in the tissues of neonatal rhesus monkeys by qRT-PCR. There may be viral inhibition in sample extraction and amplification. In

the qRT-PCR experiment, when the Ct value was greater than 35, the target gene was generally considered negative. In this experiment, a standard reference curve was obtained by measurement of standard virus RNA(Figure 1). We found that when the virus copy number of 25PFU, CT value reached 37.63. The CT value was not detected in the heart, liver, spleen, lung and blood sample. So the viral copies in the heart, liver, spleen, lung and blood may be negative or below 25PFU. There is indeed a sensitivity problem with qRT-PCR. When the number of viral RNA copies is below a certain value, the CT value will not be detected. In the future, we will improve the sensitivity of qRT-PCR by changing various conditions.



E=80.3% R^2=0.993 slope=-3.906 y-int=45.586

Figure 1. a standard reference curve