

Format for ANSWERING REVIEWERS

January 23, 2015

Dear Editor,



Please find enclosed the edited manuscript in Word format (file name: 19439-Review.doc).

Title: Pathogenicity of a currently circulating Chinese variant pseudorabies virus in pigs

Author: Qing-Yuan Yang, Zhe Sun, Fei-Fei Tan, Ling-Hua Guo, Yu-Zhou Wang, Juan Wang, Zhi-Yan Wang, Li-Lin Wang, Xiang-Dong Li, Yan Xiao, Ke-Gong Tian

Name of Journal: *World Journal of Virology*

ESPS Manuscript NO: 19439

The manuscript has been improved according to the suggestions of reviewers:

1 Format has been updated

2 Revision has been made according to the suggestions of the reviewer

Reviewed by 00503963

The current study compares the pathogenicity between PRV variant and classical Fa strain. My suggestions are described as below:

1. How about the critical/major antigenic difference or certain different in molecular level between new- and classical-PRV?

Response: *We have uploaded the PRV HN1201 genomic sequence in Genbank with access No. KP722022. However, I do not have the sequence information of classical PRV Fa strain. Therefore, the difference between these two strains remains unknown. Thank you.*

2. Fa strain is strong or weak virulent?

Response: *Fa was purchased from the Institute of China Veterinary Medicine Inspection in this study. Previous literature (Zhu L, Yi Y, Xu Z, et al. Virology Journal 2011; 8:272) reported PRV Fa strain was the earliest isolated typical strain that caused the prevalence of pseudorabies in China and caused large economic losses in the pig industry. According to our standard of virulence evaluation, Fa has the medium virulence to pigs. Thank you.*

3. The immune response is different after infection of PRV new strain?

Response: *the gE and gB antibody responses elicited by the PRV HN1201 was similar to the classical Fa strain and data was not shown in the manuscript. Thank you.*

4. In my opinion, Table 2 use “-” or “+” is not enough to express the animal situation, use

2 or 3 or 4 "+" could be more clearly to distinguish the degree of symptoms.

Response: We agree with the reviewer. Since most of pigs in group II (Fa strain) were clinical healthy, we simply distinguished them with clinical ill pigs in group I (HN1201 strain) with one "+". In the future study, we will use more "+" to describe the severity of clinical manifestations as reviewer suggested. Thank you.

5. Figure 1, normal pig organ pictures also need to show.

Response: We do not have negative control pigs which did not receive any treatment in the second animal experiment since the aim of it was to do the comparison of pathogenicity between HN1201 and Fa infection. And the gross pathology of four clinical healthy pigs in group II (Fa infection) at necropsy showed similar to the normal pig. Thank you.

6. Figure 2A and 2B, normal pig conditions need to show.

Response: Same as comment 5.

7. Figure 3, IHC staining of normal pig also need to show.

Response: Same as comment 5.

Reviewed by 00503977

Paper is focused on the characterization of a new isolate of pseudorabies virus in pigs. The paper written need a careful revision by an English native people. In addition, authors should critically read the paper and rewrite some paragraphs in order to clarify them.

Response: As reviewer suggested, we polished the English language by an English-speaking person and made the corresponding changes in the revised manuscript. Thank you.

Reviewed by 00504378

This manuscript report analysis of the pathogenicity of a new PRV variant that cannot be protected by a commonly used vaccine and causes massive economic losses in China, and comparison of the pathogenicity of this variant and classical PRV Fa stain.

Considering the importance of the disease, the manuscript is worth of publication although the comparison is very limited.

The authors showed the results that they wanted to show and provided proper discussions

However, the description of the results and discussion is not clear enough as some are mentioned as below.

The comments below are some examples and the entire manuscript need to be examined before submission of a revision.

Some comments

Lines 151-152

How come one virus strain was isolated from brain of infected pigs? The source need to be clarified.

Response: *PRV variant HN1201 was previously isolated from the brain of infected pig as previously described. Briefly, the brain sample of infected pig was homogenized and the supernatant of homogenization was subjected to 0.22µM filtration. The filtrated supernatant was inoculated on PK-15 cell monolayer until the appearance of CPE after three days. The virus was harvested after two cycles of freeze-thaw and was store at -80 until use. The description was added in the revised manuscript in line 152-156.*

Lines 199 214:

The tenses are mixed with present and past. They need to be unified. Also, this processes can be described a little bit short.

Response: *we changed the mixed tenses as reviewer suggested. Thank you.*

Lines 233-237

This sentence is redundant and can be removed or described in short

Response: *the sentences were deleted as suggested by the reviewer. Thank you.*

Lines 237-238

all pigs in two groups (group 1 and 2, 107 TCID50/pig) showed
all pigs in group I and group 2 that were inoculated with 107 TCID50 PRV HN1201 strain via intramuscular (i.m.) and intranasal (i.n.) routes, respectively showed

Response: *we made the change as reviewer suggested highlighted in line 238-240. Thank you.*

Lines 248-249

and shriving were more often observed in group 4. There was one out of five pigs in group 4 (105.0 TCID50) showed

and shriving were more often observed in group 4 (105.0 TCID50). There was one out of five pigs in group 4 showed

Response: *we made the change as reviewer suggested highlighted in line 250-251. Thank you.*

Line 253, 265

to different ages of pigs => to pigs of different ages

Response: *we made the change as reviewer suggested highlighted in line 255 and 267. Thank you.*

Line 259

pathogenic ability => pathogenicity?

Response: *we made the change as reviewer suggested highlighted in line 261. Thank you.*

Lines 265-267

a classical PRV Fa strain was used to compare with it as for the pathogenicity

a classical PRV Fa strain was compared with HN1201 for their pathogenicity.

Response: *we made the change as reviewer suggested highlighted in line 267-268. Thank you.*

Line 275

one pig appeared clinical signs => one pig showed clinical signs

Lines

Response: we made the change as reviewer suggested highlighted in line 277. Thank you.

Lines 277-279

HN1201 infection led to severe pulmonary consolidation and necrosis in the lung (Figure1A), encephalic hemorrhage in the brain (Figure 1B), and hemorrhage and necrosis in the tonsil (Figure 1C).

HN1201 infection led to severe pulmonary consolidation and necrosis in lung (Fig. 1A), encephalic hemorrhage in brain (Fig. 1B), and hemorrhage and necrosis in tonsil (Fig. 1C).

The definite article, 'the' is not necessary for the names of tissues

Figure numbers need to be abbreviated in other part, too

Response: we made the change as reviewer suggested highlighted in line 279-281. Thank you.

Lines 281-282

No other obvious pathology changes were found

No other obvious pathologic change was found

Response: we made the change as reviewer suggested highlighted in line 283-284. Thank you.

Lines 297-299. There are too many 'and'. The sentence needed to be clarified

Response: we replaced "and" with commas in this sentence. Thank you.

Line 307

and has gradually become widespread in => and has gradually spreaded in

Response: we made the change as reviewer suggested highlighted in line 308. Thank you.

Lines 346-347

Fa strain is a classical PRV virus which was used in previous studies [15]. => can be deleted

Response: the sentences were deleted as suggested by the reviewer. Thank you.

Lines 360-362

Gross pathology examination at necropsy revealed that more severe damages were observed in lung, tonsil, brain, cerebellum, and lymph node of pigs infected with HN1201 strain than Fa strain.

Gross pathology examination at necropsy revealed more severe damages in lung, tonsil, brain, cerebellum, and lymph node of pigs infected with HN1201 strain than Fa strain.

Response: we made the change as reviewer suggested highlighted in line 360-362. Thank you.

Lines 363-365

examination showed that remarkably obvious necrosis was observed in a multiple tissues such as tonsil, lung, brain, spleen and liver in HN1201-infected pigs

examination showed remarkably obvious necrosis in a multiple tissues such as tonsil, lung,

brain, spleen and liver in HN1201-infected pigs

Response: we made the change as reviewer suggested highlighted in line 363-365. Thank you.

Reviewed by 00504943

Comments to author.

The manuscript stated the pathogenicity of a new PRV variant isolated from Bartha-K61 -vaccinated pig farms with huge economic losses. Experiment design and result were clear and convincing. Only there were some language problems that should be improved, especially on the "abstract".

Response: Thank you for such positive comments from the reviewer. We did fix the language problems in the revised manuscript as reviewer suggested. Thank you.

3 References and typesetting were corrected

Thank you again for publishing our manuscript in the *World Journal of Virology*

We want to thank all reviewers for taking the time and efforts to review our manuscript and giving us a lot of valuable suggestion. We revised the manuscript and gave point-to-point feedbacks according to the reviewers' suggestions to form current version of manuscript. These changes have been highlighted in yellow in the updated version of manuscript. We believe the quality of current version of manuscript will meet to the quality for publication. Let me know if you have any further questions. Thank you.

Sincerely,

Dr. Kegong Tian

National Research Center for Veterinary Medicine

P.R. China

Editor-in-chief' comments: An outbreak of disease in economic animals always leads to economic loss and even burden in human health. Thus, the characterization of an emerging pseudorabies virus strain in vaccinated animals is an important issue and is encouraged. In accordance with those concerns raised by the reviewers, my opinion also recommended some improvement in English editing and data presentation before its formal acceptance for publication. 1. The comparison between HN1201 and Fa strain in nucleotide sequence and protein sequence should be done and present the data. The authors had read the sequence of HN1201. Since the Fa strain was reported before, it might be possible to find those sequence data. 2. Since the HN1201 strain was isolated from vaccinated animals, the epitope of vaccine and animal immune responses were crucial to the onset of disease. Thus, the data of gE/gB etc should be present. 3. In histopathological examination, the grading score is important. Thus, the presentation of 1+/2+.. is better than -/+. 4. The histological data of normal pigs should be present for clear comparison.

1. The comparison between HN1201 and Fa strain in nucleotide sequence and protein sequence should be done and present the data. The authors had read the sequence of HN1201. Since the Fa strain was reported before, it might be possible to find those sequence data.

Response: We downloaded all PRV Fa strain genes available on NCBI and compared with HN1201 in both nucleotide and protein sequences as reviewer suggested. The results were attached as appendix 1.

2. Since the HN1201 strain was isolated from vaccinated animals, the epitope of vaccine and animal immune responses were crucial to the onset of disease. Thus, the data of gE/gB etc should be present.

Response: In animal studies, piglets were died of HN1201 infection from 5dpi to 7dpi. Therefore, gE and gB antibodies cannot be detected in such early days. Thank you.

3. In histopathological examination, the grading score is important. Thus, the presentation of 1+/2+.. is better than -/+.

Response: We agreed with the reviewer and made the changes accordingly. Thank you.

4. The histological data of normal pigs should be present for clear comparison.

Response: In this study, we tried to explore the pathogenicity of HN1201 on different ages of pigs and via different inoculation routes. No control group (piglets without any treatment) was included in this study. Therefore, we cannot provide the histological data of normal pigs. Thank you.

We highly appreciated the time and efforts of reviewer put on current version of manuscript. Thank you very much.

Sincerely,

Dr. Kegong Tian