**Virologica Sinica**

**Supplementary Data**

**Isolation and oral immunogenicity assessment of porcine epidemic diarrhea virus NH-TA2020 Strain: one of the predominant strains circulating in China from 2017 to 2021**

Xiaowen Li a, c, 1,Yang Li a, c, 1, Jiapei Huang a, c, Yali Yao b, Wenying Zhao b, Yunjing Zhang b, Jie Qing a, c, Jing Ren d, Zhong Yan a, Zewei Wang a, c, Xiaofang Hu a, c, Duli Kang e, Hongqiang Liu e, Zhichun Yan a, c, \*

**a** New Hope Liuhe Co., Ltd., Chengdu, 610041, China.

**b** National Research Center for Veterinary Medicine, Luoyang, 471000, China;

**c** Shandong New Hope Liuhe Agriculture and Animal Husbandry Technology Co., Ltd. (NHLH Academy of Swine Research), Dezhou, 253034, China.

**d** Swine Health Data and Intelligent Monitoring Project Laboratory, Dezhou University, Dezhou, 253011, China.

**e** Pulike Biological Engineering Inc., Luoyang, 471000, China.

\*Corresponding author:

Email: Jasonynh@126.com (Z. Yan)

ORCID: orcid.org/0000-0001-7297-9578

1 Xiaowen Li and Yang Li contributed equally to this work.

Table S1 Primers information used in this study.

|  |  |  |
| --- | --- | --- |
| **Primers** | **Sequences (5’-3’)** | **Product Length/bp** |
| PEDV-F | TTCGGTTCTATTCCCGTTGATG | 663 |
| PEDV-R | CCCATGAAGCACTTTCTCACTATC |
| TGEV-F | TTACAAACTCGCTATCGCATGG | 528 |
| TGEV-R | TCTTGTCACATCACCTTTACCTGC |
| PDCoV-F | GACCCTAAATCTGCCGTTAGAG | 543 |
| PDCoV-R | TGTTGGAGAGGTGAATGCTATG |
| PoRV-F | CCCCGGTATTGAATATACCACAGT | 333 |
| PoRV-R | TTTCTGTTGGCCACCCTTTAGT |
| PEDV-S1-F | TTTGTGGCTTTTCTAATC | 2286 |
| PEDV-S1-R | CACCTATGTTACTATACACC |
| PEDV-N-F | GAGGGTGTTTTCTGGGTTG | 226 |
| PEDV-N-R | TGCCTCTGTTGTTACTTGG |
| PEDV-S-1-F | ACAATGTTCTGTACCAGTGTT | 1300 |
| PEDV-S-1-R | TTAAACCTCAGAGCCTCTGGTGCA |
| PEDV-S-2-F | TGTCCAATGATTCCACTTTGG | 1588 |
| PEDV-S-2-R | CTCATACTAAAGTTGGTGGG |
| PEDV-S-3-F | ATTCTAATGATGGCTCTAATTG | 1605 |
| PEDV-S-3-R | AAACATCTAGAGAAAGACTTG |
| PEDV-S-4-F | TTGAGAGTTGTGTGGTCACC | 1105 |
| PEDV-S-4-R | AACAATTTGATGTTGCGAAAAG |

Table S2 Viral shedding of NH-TA2020 strain infected gilts.

|  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Gilts NO.** | **Weeks after immunization** | | | | | | | | |
| 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 |
| 1 | + | + | + | + | - | - | - | - | - |
| 2 | + | + | + | + | + | - | - | - | - |
| 3 | + | + | + | + | - | - | - | - | - |
| 4 | + | + | + | + | + | - | - | - | - |
| 5 | + | + | + | - | + | - | - | - | - |
| 6 | + | + | + | + | - | - | - | - | - |

Six gilts were challenged with 2×107 TCID50 NH-TA2020 viruses orally 60 days before delivery (corresponding to group A and group C of Table 3). Disinfection was performed every day and PEDV RNA was detected every week by qPCR. Six gilts delivered at the 8th week. +: positive; -: negative.

Table S3 The GenBank accession numbers for nucleotide sequences.

|  |  |
| --- | --- |
| **PEDV strains** | **Accession NO.** |
| CH-JX-YT3-2021 | ON168750 |
| CH-JS-YC349-2021 | ON168751 |
| CH-JS-XZ672-2020 | ON168752 |
| CH-HeB-XJXM7-2020 | ON168753 |
| CH-GX-XZC304-2021 | ON168754 |
| CH-JX-XRC186-2021 | ON168755 |
| CH-SD-XJ-2020 | ON168756 |
| CH-HeB-TS75-2020 | ON168757 |
| CH-HeN-TQ1-2021 | ON168758 |
| CH-HeN-RZF8-2020 | ON168759 |
| CH-JS-RG-2020 | ON168760 |
| CH-ShaanXi-PC-2021 | ON168761 |
| CH-GX-IM253-2021 | ON168762 |
| CH-SD-LQL6-2020 | ON168763 |
| CH-GX-LP74-2021 | ON168764 |
| CH-SD-LY241-2020 | ON168765 |
| CH-SD-LYXJA68-2020 | ON168766 |
| CH-SD-LYGZG250-2020 | ON168767 |
| CH-SD-LYDWJ01-2020 | ON168768 |
| CH-SD-LX9-2020 | ON168769 |
| CH-GX-LB58-2021 | ON168770 |
| CH-LN-KPE18-2021 | ON168771 |
| CH-LN-KPA4-2021 | ON168772 |
| CH-JX-6-2021 | ON168773 |
| CH-Shanxi-HR301-2020 | ON168774 |
| CH-Shanxi-HRP3-2021 | ON168775 |
| CH-HuB-900-2020 | ON168776 |
| CH-HuN-HN839-2021 | ON168777 |
| CH-LN-HS23-2020 | ON168778 |
| CH-Shaanxi-HY1-2021 | ON168779 |
| CH-SC-GY7-2021 | ON168780 |
| CH-JS-DHA426-2020 | ON168781 |
| CH-SD-BZC53-2021 | ON168782 |
| CH-GS-BYC195-2021 | ON168783 |
| CH-HeN-AY112-2021 | ON168784 |
| CH-SC-AYC500-2020 | ON168785 |
| CH-SC-SZ1704-2017 | ON168786 |
| CH-SD-XJ1710-2017 | ON168787 |
| CH-SD-XJ1704-2017 | ON168788 |
| CH-SD-XJ1703-2017 | ON168789 |
| CH-SD-LY1803-2018 | ON168790 |
| CH-SD-LY1711-2017 | ON168791 |
| CH-SD-LY1702-2017 | ON168792 |
| CH-SD-LS1803-2018 | ON168793 |
| CH-SD-LS1712-2017 | ON168794 |
| CH-SD-LS1702-2017 | ON168795 |
| CH-SD-LQ1803-2018 | ON168796 |
| CH-SD-LQ1712-2017 | ON168797 |
| CH-SD-LQ1802-2018 | ON168798 |
| CH-SD-LQ1706-2017 | ON168799 |
| CH-SD-LK1801-2018 | ON168800 |
| CH-HeB-HS1711-2017 | ON168801 |
| CH-SD-GM1701-2017 | ON168802 |
| NH-TA2020 | ON168803 |
| CH-HeB-RY-2020 | ON168804 |
| CH-LN-KJW-2021 | ON168805 |
| CH-SD-SX-2020 | ON168806 |
| CH-GX-LP-2021 | ON168807 |
| CH-AH-XX-2021 | ON168808 |
| CH-HeB-HH-2021 | ON168809 |
| CH-GS-GS1-2021 | ON168810 |
| CH-HuN-HY-2021 | ON168811 |
| CH-HuB-LH-2021 | ON168812 |
| CH-LN-2021 | ON168813 |
| CH-LN-SY-2021 | ON168814 |
| CH-IM-TL-2020 | ON168815 |
| CH-SD-DY-2021 | ON168816 |
| CH-HeB-HS-2021 | ON168817 |
| CH-AH-WH-2021 | ON168818 |
| CH-HuB-GL-2021 | ON168819 |
| CH-IM-ZN-2021 | ON168820 |
| CH-BJ-2021 | ON168821 |
| CH-GS-2021 | ON168822 |
| CH-GS-LZ-2021 | ON168823 |
| CH-SD-LS-2020 | ON168824 |
| CH-HeB-HHY-2020 | ON168825 |
| CH-HuB-LQ-2020 | ON168826 |
| CH-HeB-QHY-2020 | ON168827 |
| CH-HeB-FC-2020 | ON168828 |
| CH-HeB-XQH-2020 | ON168829 |
| CH-SD-XJYF-2018 | ON168830 |
| CH-SD-XJ-2018 | ON168831 |
| CH-HeB-HS-2018 | ON168832 |
| CH-HeB-FHS-2018 | ON168833 |
| CH-SD-XJ-2017 | ON168834 |
| CH-SD-LY-2019 | ON168835 |
| CH-SC-QS-2019 | ON168836 |
| CH-SC-KQTP-2019 | ON168837 |
| CH-SC-XP-2019 | ON168838 |
| CH-SC-QQ1-2018 | ON168839 |
| CH-SC-RC-2018 | ON168840 |
| CH-SC-SW-2018 | ON168841 |
| CH-SC-YM-2018 | ON168842 |
| CH-SC-JY-2019 | ON168843 |
| CH-SC-SZ-2019 | ON168844 |
| CH-SC-QQ-2019 | ON168845 |
| CH-SC-TZ-2019 | ON168846 |
| CH-SC-JH-2020 | ON168847 |
| CH-SC-DYS5-2020 | ON168848 |
| CH-SC-DY1-2018 | ON168849 |
| CH-SC-SZF1-2018 | ON168850 |
| CH-SC-TZ-2018 | ON168851 |
| CH-SC-XP1-2018 | ON168852 |
| CH-SC-SZ4-2020 | ON168853 |



Figure S1. The isolation and identification of PEDV NH-TA2020 strain. **A** Detecting the causative agents of the diarrhea material. The small intestine of a piglet with diarrhea was collected. The potential diarrhea agents, including TGEV (lane 4), PDCoV (lane 5), PoRV (lane 6) were detected using specific primers listed in Supplementary Table S1 by PCR, respectively. The PEDV positive sample control (lane 1), the negative sample control (lane 2), the negative nucleic acid extraction control (lane 3), and the diarrhea material (lane 7) were detected using specific primers of PEDV-F and PEDV-R by PCR. M, DL 2000 Marker. **B** The complete sequence of PEDV S gene was amplified in four reactions using specific primers by PCR. 1, fragment of S-1; 2, fragment of S-2; 3, fragment of S-3; 4, fragment of S-4.



Figure S2. NH-TA2020 strain exhibited strong pathogenicity in gilts. Six gilts were challenged with 2×107 TCID50 NH-TA2020 viruses orally 60 days before delivery (corresponding to group A and group C of Table 3). The clinical symptoms including depression, diarrhea and anorexia were found in all six gilts within three days after immunization. The negative control gilts were treated with DMEM and no clinical symptoms of PED was observed.