Experimental pig study confirms the high virulence of the recently emerged PRRSV 1-4-4 L1C variant strain
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Introduction
Since October 2020, high mortality and morbidity of pigs associated with PRRSV have been observed in U.S. swine farms. ORF5 sequence analysis suggested that the detected PRRS viruses from these cases had a 1-4-4 RFLP pattern and formed a distinct cluster within the Lineage 1C; these PRRS viruses were tentatively referred to as “1-4-4 L1C variant” or “L1C variant”. However, no unequivocal experimental data was available to confirm the perception that the 1-4-4 L1C variant is more virulent than other PRRSV strains. The objective of this study was to experimentally characterize the pathogenicity and transmissibility of PRRSV 1-4-4 L1C variant strain in comparison with other PRRSV strains.

Materials and Methods
In 2020-2021, most RFLP 1-4-4 PRRSVs belonged to L1A, L1C variant, L1C non-variant, and L1H sublineages in the U.S. Thus, in this study, four 1-4-4 PRRSV strains (L1C variant, L1C non-variant, L1A, and L1H) and one previously described virulent PRRSV 1-7-4 L1A strain were included for comparison. Seventy-two 3-week-old PRRSV-naive pigs were blocked by weight and randomly divided into 6 groups with 12 pigs per group. Forty-eight pigs (8/group) were for inoculation and 24 pigs (4/group) served as contact pigs. After one-week acclimation, 8 pigs in each group were inoculated with the corresponding virus (10^6 TCID50/pig, based on titration in ZMAC cells) or negative media intramuscularly and intranasally. At 2 days post inoculation (DPI), contact pigs were added to the pen adjacent to the inoculated pigs in each room. Daily temperature and clinical signs were recorded. Serum and oral fluids were collected at 0, 2, 4, 7, 10, 14, 21 and 28 DPI. Pigs were necropsied at 10 & 28 DPI.

Results
The 1-4-4 L1C variant-inoculated pigs became more anorectic and lethargic, had higher mortality, had higher fraction of pigs with fever (>40°C) during 0-10 DPI, and had significantly higher average temperature than other virus-inoculated groups at several time points. The mean average daily weight gain in 1-4-4 L1C variant-inoculated group was numerically higher but not statistically significant compared to other virus-inoculated groups. The 1-4-4 L1C variant-inoculated group had significantly higher viremia levels compared to all other virus-inoculated groups at 2 DPI. The same trend of viremia level was found in contact pigs at 2 days post contact (DPC). 4/4, 2/4, 2/4, 0/4, and 2/4 Contact pigs in the 1-4-4 L1C variant, 1-4-4 L1C non-variant, 1-4-4 L1A, 1-4-4 L1H, and 1-7-4 L1A groups became viremic at 2 DPC, respectively, implying the potential higher transmissibility of the 1-4-4 L1C variant virus. There were more severe gross lung lesions (percentage of interstitial pneumonia) in the 1-4-4 L1C variant-inoculated group compared to others except for the 1-7-4 L1A group at 10 DPI. The differences of microscopic lung lesion score and PRRSV IHC score in lung tissues at 10 DPI were not statistically significant between virus-inoculated groups.

Discussion and Conclusion
This study provides experimental data in weaned pigs regarding the clinical impact, pathogenicity, and transmissibility of the newly emerged 1-4-4 L1C variant strain, along with comparisons with other PRRSV strains. The findings from this experimental study align with what field veterinarians observed for the L1C variant outbreak and confirm that the 1-4-4 L1C variant is highly virulent in weaned pigs. The higher number of contact pigs becoming viremic at 2 days post contact implies that the L1C variant strain may have higher transmissibility than other PRRSV strains. However, it is complicated to assess virus transmissibility accurately, and the result needs to be confirmed with a study involving more pigs. Future studies are needed to investigate the protective efficacy of PRRS MLV vaccines against the 1-4-4 L1C variant strain.

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