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Evaluation of the Efficacy of an Attenuated Live Vaccine against Highly Pathogenic Porcine Reproductive and Respiratory Syndrome Virus in Young Pigs

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Highly pathogenic porcine reproductive and respiratory syndrome virus (HP-PRRSV) is characterized by high fever and high mortality in pigs of all ages and has severely affected the pork industry of China in the last few years. An attenuated HP-PRRSV strain, TJM, was obtained by passaging HP-PRRSV strain TJ on MARC-145 cells for 92 passages. Porcine reproductive and respiratory syndrome virus (PRRSV)- and antibody-free pigs were inoculated intramuscularly with TJM ($10^{5.0}$ 50% tissue culture infective doses [$TCID_{50}$]) and challenged at 28, 60, 120, and 180 days postimmunization (dpi). The results showed that 5/5, 5/5, 5/5, and 4/5 immunized pigs were protected from the lethal challenge and did not develop fever and clinical diseases at each challenge, respectively. Compared to control pigs, vaccinated pigs showed much milder pathological lesions and gained significantly more weight ($P < 0.01$). Sequence analysis of different passages of strain TJ showed that the attenuation resulted in a deletion of a continuous 120 amino acids (aa), in addition to the discontinuous 30-aa deletion in the nsp2 region. The analysis also demonstrated that the 120-aa deletion was genetically stable *in vivo*. These results suggested that HP-PRRSV TJM was efficacious against a lethal challenge with a virulent HP-PRRSV strain, and effective protection could last at least 4 months. Therefore, strain TJM is a good candidate for an efficacious modified live virus vaccine as well as a useful molecular marker vaccine against HP-PRRSV.

Porcine reproductive and respiratory syndrome (PRRS) is characterized by respiratory distress in piglets and reproductive failure in sows (3, 6). The disease was detected in North America in 1987 (9, 11) and in Europe in 1990 (25), and it has now become a well-recognized global swine disease (1, 6, 10, 23, 35). PRRS has devastated the swine industry by causing tremendous economic losses throughout the world and is now considered to be one of the most important diseases in countries with intensive swine industries (16, 20).

The causative agent, PRRS virus (PRRSV), is a positive-strand RNA virus which belongs to the family *Arteriviridae*, together with lactate dehydrogenase-elevating virus (LDV) of mice, equine arteritis virus (EAV), and simian hemorrhagic fever virus (SHFV) (5, 31). PRRSV isolates can be divided into the European (EU) and the North American (NA) genotypes, which are genetically and antigenically different (4, 21, 22). Currently, a limited number of PRRSV vaccines, either modified live or killed vaccines, are commercially available and efficacious. These vaccines contain a single PRRSV strain and are not fully protective against genetically diverse field strains in most cases (17). In addition, outbreaks of atypical or acute PRRS, a severe form of PRRS characterized by abortion and high mortality in pregnant sows, were reported for vaccinated pigs (2, 18). Since May 2006, a highly virulent form of PRRSV (highly pathogenic PRRSV [HP-PRRSV]), characterized by high fever and high mortality in pigs of all ages, has severely affected the pork industry of China (14, 32, 34). Therefore, there is a strong need for a new vaccine to be developed against emerging HP-PRRSV strains.

HP-PRRSV strain TJ was originally isolated from a piglet that died of a "high fever" in Tianjin, China, in 2006, and it had the same characteristics as those of other HP-PRRSV strains observed in China (13). HP-PRRSV strain TJ was culturally passaged on MARC-145 cells for attenuation so that it could be used for the development of a modified live virus (MLV) vaccine. The purposes of this study were to attenuate a virulent HP-PRRSV TJ strain, genetically characterize the attenuated (92nd passage of strain TJ) HP-PRRSV strain TJM, and evaluate its virulence and immunogenicity in host animals.

MATERIALS AND METHODS

Cells and viruses. The MARC-145 cell line (derived from African green monkey kidney cells) was employed for viral propagation and titration. PRRSV strain TJ (GenBank accession no. EU860248) was isolated and maintained in our laboratory as previously described (13).

Viral culturing and attenuation. PRRSV strain TJ was continuously passaged on MARC-145 cells and plaque cloned every 5 to 10 passages. For plaque cloning, the virus suspension was serially 10-fold diluted, and 200 μl of virus was inoculated onto MARC-145 monolayers in 6-well microtiter tissue culture plates. After 1 h of incubation at 37°C, the cell monolayers were then overlaid with minimal essential medium (MEM) (2×) containing 2% low-melting agarose (Cambrex, Rockland, ME) and 4% fetal bovine serum and then incubated for 72 h to 96 h at 37°C with 5% CO_2. Single plaques were selected, and the viruses were further passaged. The 92nd passage of PRRSV strain TJ was harvested and designated HP-PRRSV strain TJM, which was characterized and evaluated in this study.

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