I prefer:

□ ORAL presentation

POSTER presentation

**Generation and characterization of chimeric TMUVs viruses containing prM and E genes of JEV**

Bangfeng Xu1\*, Xingpo Liu1, Dawei Yan1, Qiaoyang Teng1, Chunxiu Yuan1, Zhifei Zhang1, Qinfang Liu1, Zejun Li1

\*lead presenter

1 [xubangfeng@shvri.ac.cn](mailto:xubangfeng@shvri.ac.cn), Shanghai Veterinary Research Institute, Chinese Academy of Agricultural Sciences, China

**Abstract:** Since its outbreak in 2010, Tembusu virus (TMUV) has spread widely throughout China and Southeast Asia, causing significant economic losses to the poultry industry. In 2018, an attenuated vaccine called FX2010-180P (180P) was licensed for use in China. The 180P vaccine has demonstrated its immunogenicity and safety in mice and ducks. The potential use of 180P as a backbone for flavivirus vaccine development was explored by replacing the pre-membrane (prM) and Envelope (E) genes of the 180P vaccine strain with those of Japanese encephalitis virus (JEV). Two chimeric viruses, 180P/JEV-prM-E and 180P/JEV-prM-ES156P with an additional E protein S156P mutation were successfully rescued and characterized. Growth kinetics studies showed that the two chimeric viruses replicated to similar titers as the parental 180P virus in cells. Animal studies also revealed that the virulence and neuroinvasiveness of the 180P/JEV-prM-E chimeric virus was decreased in mice inoculated intracerebrally (i.c.) and intranasally (i.n.), respectively, compared to the wild-type JEV strain. However, the chimeric 180P/JEV-prM-E virus was still more virulent than the parent 180P vaccine in mice. Additionally, the introduction of a single ES156P mutation in the chimeric virus 180P/JEV-prM-ES156P further attenuated the virus, which provided complete protection against challenge with a virulent JEV strain in the mouse model. These results indicated that the FX2010-180P could be used as a promising backbone for flavivirus vaccine development.

**Background/Objective:** TMUV, a member of the Flavivirus genus in the Flaviviridae family. In 2010, an outbreak of TMUV occurred in China, resulting in major clinical symptoms such as growth retardation, severe reduction in egg production, and neurological symptoms. Since then, the virus has rapidly spread among duck farms in China and Southeast Asia, becoming endemic. To control the spread and prevalence of TMUV in duck population, an attenuated vaccine FX2010-180P (180P) was developed through serial passaging of a wildtype TMUV-FX2010 on chicken embryo fibroblasts (CEF). Subsequent studies showed that the 180P was attenuated in mice and ducks, with no clinical symptoms or tissue damage observed in ducks infected by a high-dose inoculation. Moreover, a low-dose 180P elicited good immunogenicity in ducks and provided complete protection against challenge with a virulent strain. which indicated that the 180P was an ideal attenuated vaccine strain. As a result, the 180P vaccine was licensed in China in 2018 and is now widely used in ducks to prevent the TMUV infection. Therefore, in order to prevent other flavivirus from infecting poultry, we explored whether TMUV attenuated vaccine strain can be used as a backbone to rapidly develop other flavivirus (JEV) attenuated vaccines.

**Methods:** To generate the templates for PCR-based virus rescue, four plasmids p180PT7-1-976, p180PE957-2459, p180P2433-3831, and p180P3656-10991 containing the overlapping fragments of the FX2010-180P were generated, and the full-length cDNA of 180P with T7 promoter was produced by fusion-PCR using the four overlapping fragments. To generate the infectious viral RNAs, the cDNAs were transcribed in vitro using mMESSAGEmMACHINE® T7 Kit and purified by lithium chloride precipitation. The RNAs were then transfected into BHK-21 cells at 70-80% confluency, cultured in T25 cm2 flasks, with an amount of 5 μg using Lipofectamine LTX& Plus Reagent (Invitrogen, Carlsbad, CA, USA). The cell culture medium was changed to DMEM containing 2% FBS at 6 h post-transfection. The virus released into the supernatant was collected when 70-80% transfected cells start showing apparent cytopathic effects (CPEs). To rescue the 180P/JEV-prM-E and 180P/JEV-prM-ES156P viruses, the plasmids p180PT7-1-455, pJEV-prM-E, and pJEV-prM-ES156P were constructed, and plasmids p180PT7-1-976 and p180PE957-2459 were replaced by the p180PT7-1-455, pJEV-prM-E, and pJEV-prM-ES156P plasmids during rescues, respectively. The viral replication of the rescue viruses was determined by the growth kinetics curve and the ability of plaque formation. The virulence and neuroinvasiveness of parental and chimeric viruses were tested in mice. To explore the efficacy of 180P/JEV-prM-ES156P virus, the vaccination-challenge experiment was conducted in mouse model.

**Results:** The sequence analysis showed that the two chimeric viruses 180P/JEV-prM-E and 180P/JEV-prM-ES156P were successfully rescued in the background of the 180P. The growth kinetics results suggested that the JEV prM-E is compatible with the backbone of 180P, and did not affect the replication abilities of chimeric viruses significantly. Animal studies also revealed that the virulence and neuroinvasiveness of the 180P/JEV-prM-E chimeric virus was decreased in mice inoculated intracerebrally (i.c.) and intranasally (i.n.), respectively, compared to the wild-type JEV strain. However, the chimeric 180P/JEV-prM-E virus was still more virulent than the parent 180P vaccine in mice. Additionally, the introduction of a single ES156P mutation in the chimeric virus 180P/JEV-prM-ES156P further attenuated the virus, which provided complete protection against challenge with a virulent JEV strain in the mouse model. These results indicated that the FX2010-180P could be used as a promising backbone for flavivirus vaccine development.

**Conclusion:** In this study, we demonstrated that TMUV FX2010-180P vaccine strain can be used as a backbone to develop JEV attenuated vaccine candidate, which provided a new vector tool for the development and preparation of flavivirus vaccines.

**Keywords:** TMUV, JEV, Flavivirus, Attenuated vaccines, Virulence