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**Complete genome sequence and construction of an infectious bacterial artificial chromosome clone of a virulent duck enteritis virus strain XJ**

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**Abstract:**

**Background/Objective:** Duck plague, caused by Duck Enteritis Virus (DEV), is an acute, contagious, septicemic infectious disease that result in substantial economic losses for the global poultry industry. The limited adaptability of DEV to passage cells and the scarcity of commercial antibodies for virus research have led current investigation to primarily focus on gene function, thereby constraining the exploration of its pathogenic and molecular mechanisms. In 2021, a highly virulent strain of duck enteritis virus (DEV), designated as DEV XJ, was isolated from Zhejiang, China. To further investigate the mutations and gene functions of the DEV, this study conducted whole genome sequencing of the newly isolated virulent strain DEV XJ and generated a recombinant virus XJ BAC with similar biological characteristics to the parental strain. Furthermore, the *US3*, *LORF3*, *UL21*, and *UL36* genes were individually deleted using two-step RED recombination approach based on the infectious BAC clone. The functional roles of the above-mentioned mutant genes were preliminarily explored through the analysis of their *in vitro* biological characteristics.

**Methods:** The complete genome was sequenced for comprehensive analysis. To understand the gene function and the molecular mechanism of the DEV XJ strain, a full length infectious clone of DEV XJ was constructed using homologous recombination. To investigate the potential impact of mutations, the *US3*, *LORF3*, *UL21*, and *UL36* genes were individually deleted based on the infectious BAC clone using two-step RED recombination approach.

**Results:** The complete genome of DEV XJ, spanning 162234-bp with 78 predicted open reading frames (ORFs), was sequenced. While showing relative homology to the DEV CV strain, DEV XJ exhibited distinctions in 38 ORFs, including various immunogenic and virulence-related genes. Amino acid variation analysis, focusing on UL6 and LORF3, indicated that high degree of homology between DEV XJ and the 2085 strain from Europe, as well as the DEV DP-AS-Km-19 strain from India. Subsequently, a full-length infectious bacterial artificial chromosome clone (BAC) of DEV XJ was successfully constructed to delve into the pathogenic mechanisms of this virulent strain. XJ BAC demonstrated substantial similarity to the parental DEV XJ in both *in vitro* growth properties and the induction of typical pathogenic symptoms in sheldrakes. Furthermore, the *US3*, *LORF3*, *UL21*, and *UL36* genes were individually deleted using two-step RED recombination approach based on the infectious BAC clone. Our findings revealed that the UL21 and UL36 genes play crucial roles in viral proliferation. Although the *US3* and *LORF3* genes were dispensable for viral replication and cell-to-cell transmission *in vitro*, they attenuated the replication and transmission efficiency of DEV compared to the WT.

**Conclusion:** This study accomplished the whole-genome sequencing of a clinically virulent DEV strains and the successful construction of an infectious DEV XJ clone. Moreover, the functional roles of the above-mentioned mutant genes were preliminarily explored through the analysis of their *in vitro* biological characteristics.

**Keywords:** Phylogenetic analysis; BACs; Pathogenicity; Duck enteritis virus.