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GENOME ANNOUNCEMENT

Complete Genome Analysis of Three Live Attenuated Rinderpest Virus Vaccine Strains Derived through Serial Passages in Different Culture Systems

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The genomes of three South Korean Rinderpest virus vaccine strains (L72, LA77, and LA96) were analyzed in order to investigate their genetic variability. These three vaccine strains were all derived from the same virus strain origin (Fusan) through repeated passages in different culture systems. The full genome length of the three strains was 15,882 nucleotides, and the sequence similarity between the three South Korean RPV strains at the nucleotide level was 98.1 to 98.9%. The genetic distance between Nakamura III, L72, LA77, LA96, and LATC06 and the Kabete strain was greater than that between the Fusan and Kabete strains for the P, V, and C genes. The difference in pathogenicity among these strains might be due to the V gene, which has a positive (>1) selection ratio based on the analysis of synonymous (*dS*) and nonsynonymous (*dN*) substitution rates (*dN*/*dS* ratio [ω]).

Rinderpest virus (RPV) belongs to the genus *Morbillivirus* within the family *Paramyxoviridae*. It is related to the measles virus, canine and phocid (seal) distemper viruses, the cetacean morbillivirus, and the peste des petits ruminants virus (2). The L72 strain, derived from the Fusan strain (3), was passaged in rabbits. The LA77 strain, derived from the L72 strain, was passaged in chicken embryos. The LA96 strain, derived from the LA77 strain, was passaged in chicken embryo fibroblast cells. The genome sequences of the Kabete, RBOK, and LATC06 RPV strains have been analyzed (1, 5). In recent publications, the complete genomic sequences of the lapinized Nakamura III strain and the Fusan strain, which are the classical isolates from cattle in Asia, have been reported (3).

Reverse transcription of RNA extracted from the three strains was performed using a cDNA synthesis kit (TaKaRa Bio, Inc., Japan) and either oligo(dT) or random primers. PCR was used to synthesize cDNA using overlapping fragments of 2 to 3 kb. Both strands were amplified using various primers spaced approximately 500 bases apart along the entire genome and sequenced using a 3730XL capillary DNA sequencer (ABI, United States).

The amino acid sequence similarity between Fusan and other strains was lowest (<90%) within the P, C, and V proteins. The H protein of strain L72 showed 93.4% and 100% homology at the amino acid level with the Fusan and Nakamura III H proteins, respectively. The analysis of RPVs using PAL2NAL, which automatically calculates synonymous (*dS*) and nonsynonymous (*dN*) substitution rates using the codeml program in PMAL, showed that five genes (N, M, F, H and L) have low ω (*dN*/*dS*) ratios, but the P, C, and V genes have high ω ratios. The ω ratios of the V genes among the RPVs shown were considerably high, ranging from 0.83 to 1.49.

A Bayesian tree (-lnL = 6,643.2520, GTR + I + G model) of N genes for 15 RPV strains contained four lineages according to geographical region. The Africa-1 lineage contained the Kabete O and RBOK vaccine strains, whereas the Africa-2 lineage included the Egypt/84, Buffalo, Sokot, RGK1, and RBT1 strains. The Middle East lineage contained Saudi/81 and Kuwait 82/1, and the Asia lineage had the same topology in the phylogenetic tree of the Nakamura III, Fusan, L72, LA77, LA96, and LATC06 strains.

There has been no reemergence of rinderpest anywhere in the world since it was last detected in Kenya in 2001. This outcome of the Global Rinderpest Eradication Programme represents an astounding achievement for veterinary science (4). Although this virus has now disappeared from nature, the present study shows that genetic variability may occur when the vaccine virus strain that has been attenuated through the animal passage method would be further adapted into a cell culture system. This information can be added to the baseline data pertaining to RPVs of Asian lineage.

Nucleotide sequence accession numbers. The complete genome sequences of the South Korean RPV strains L72, LA77, and LA96 have been deposited in GenBank under accession no. JN234008 to JN234010.

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REFERENCES

1. **Baron MD, Kamata Y, Barras V, Goatley L, Barrett T.** 1996. The genome sequence of the virulent Kabete 'O' strain of rinderpest virus: comparison with the derived vaccine. J. Gen. Virol. **77**:3041–3046.
2. **Barrett T, et al.** 1993. Dolphin and porpoise morbilliviruses are genetically distinct from phocine distemper virus. Virology **193**:1010–1012.
3. **Fukai K, Morioka K, Sakamoto K, Yoshida K.** 4 June 2011. Characterization of the complete genomic sequence of the rinderpest virus Fusan strain cattle type, which is the most classical isolate in Asia and comparison with its lapinized strain. Virus Genes [Epub ahead of print.] doi:10.1007/s11262-011-0630-8.
4. **Roeder PL.** 2011. Rinderpest: the end of cattle plague. Prev. Vet. Med. **102**:98–106.
5. **Yeh JY, et al.** 2011. Genetic characterization of the Korean LATC06 rinderpest vaccine strain. Virus Genes **42**:71–75.