DEVELOPMENT OF AN ATTENUATED NEWCASTLE DISEASE VIRUS FROM A HIGH PATHOGENIC VIRUS BY RECOMBINATION OF GENOMES

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Introduction. The Newcastle disease is one of the most important infectious diseases in avian industry. This disease is caused by a Paramyxoviridae family member of the genus Avulavirus named Newcastle disease virus (NDV), of this virus 15 genotypes has been described [1]. NDV is an enveloped RNA virus of negative sense. It´s genome contains six genes in order 3'→5' NP-P-M-F-HN-L. Of these genes, F and HN codify for membrane proteins and play an important role in the pathogenesis process. Additionally, five internal amino acids of the F protein, are the principal determinant of pathogenicity. On the other hand, these two proteins are the immunogenic proteins and are responsible of the immune response in form of antibodies. For a better vaccine protection, the vaccine virus should be very similar to field. If the virus are of a genotype different the efficacy of vaccine protection decrees. For this reason, in recent years several authors recommend vaccine according to the genotype of the field virus [2].

In this work, we describe the construction of a recombinant virus by exchange of genes encoding F and HN proteins of a NDV genotype II by F and HN proteins of a genotype V.

Methods. We use two virus for exchange, the genotype II virus was the strain "Lasota", the more used virus for vaccine development. The genotype V virus was the strain P05 previously described by our research group [3]. The Lasota strain was amplified by RT-PCR to obtain the complete genome of the NDV into 8 segments. All these segments were assembled to form the full length cDNA genome. On the other hand, the F and HN genes were amplified into a unique segment of 3.7 kb. For replacement of a partial segment of the F gene and the complete HN gene, the full length cDNA genome of Lasota strain and the P05 strain segment were double digest with SpeI–NotI restriction enzymes. Then the segment P05 was ligated into the full length cDNA genome of Lasota strain. Finally, the new plasmid with the backbone of Lasota strain with the partial F and HN genes of the P05 strain was used for rescue of the recombinant Newcastle disease virus (fig. 1). After of rescue the virus the virulence of the new strain named LSP05 was determinate according to the intracranial pathogenicity index (ICPI) and the mean death time (MDT) of the chicken embryos.

Results. After of rescue the virus we compare the pathogenicity of the new virus and compare with the strain lasota and P05. The results are shown in table I. According to this results the new recombinant virus is lentogenic.

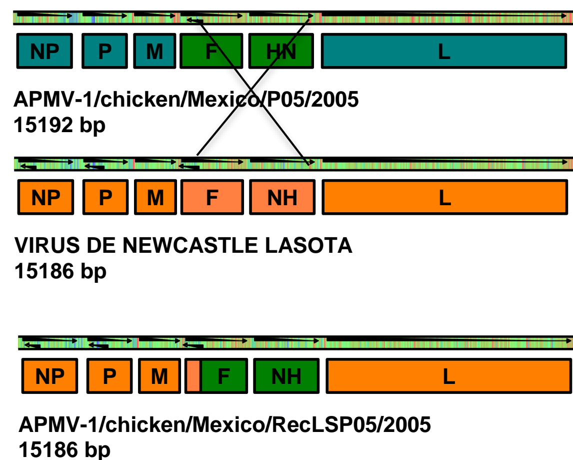

Fig.1 Map of the gene exchange between the NDV's Lasota and P05 strains. In the lower image the map of the new recombinant virus named LSP05.

TABLE I. Virulence of the two virus

	Lasota	P05	RecP05
ICPI	0.2	1.99	0.28
MDT	> 96	36 h	> 96

Conclusions. In this work we develop a new strategy for develop new recombinant virus by replace segments of antigenic proteins. This knowledge could be used for develop viral vaccines from any genotype.

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