DEVELOPMENT OF A RECOMBINANT VIRUS OF A HIGH PATOGENIC NEWCASTLE DISEASE VIRUS

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Introduction. The Newcastle disease is one of the most important infectious diseases of poultry. Newcastle disease is caused by the Newcastle disease virus (NDV), an enveloped RNA virus from the Paramyxoviridae family, genus Avulavirus [1]. Viruses are classified into three pathotypes according to their pathogenicity index, based on chicken embryo mortality: (1) lentogenic viruses that cause mild symptoms with no mortality, (2) velogenic viruses which approach 100 % mortality, and (3) mesogenic viruses which exhibit a moderate pathogenicity with mortality rates ranging from 30 to 50 % with pronounced symptoms. The NDV genome consists of a single stranded, non-segmented, negative-sense, RNA. The genome contains six genes: NP-P-M-F-HN-L, of which F is the determinant of pathogenicity [1].
In this work, we describe the isolation of a velogenic strain of NDV named APMV-1/Mexico/P05/2005 (P05) [2] by *Investigación Aplicada S.A. de C.V*. (IASA) and the use of reverse genetic for obtain a recombinant lentogenic virus by modify the amino acids sequence of the F protein by site direct mutagenesis.

Methods. The virus was isolated from internal organs of a hen exhibiting severe symptoms of the disease. We used the isolation method extensively described by Alexander in 1989. The virulence of the P05 strain was determinate according to the intracranial pathogenicity index (ICPI) and the mean death time (MDT) of the chicken embryos [2]. To obtain the recombinant virus, we did reverse genetic; briefly, from the genomic RNA of the NDV (RNA genome size of 15,192 pb), we use RT-PCR for obtain genomic cDNA. The genome was amplify in 8 cDNA segments and after assemble for obtain the full length cDNA genome in a unique plasmid. Previously to assembly, the PCR product containing the F gene was modified using site directed mutagenesis. The nucleotide sequence replaced was the corresponding to the cleavage site of the F protein by proteases effect. The amino acid sequence of the high virulent virus is RRQKR. After mutagenesis the sequence was changed to GRQGR. Finally, the plasmid containing the full length cDNA genome of NDV was used for rescue of the recombinant virus.

Results. We isolate a high pathogenic virus with a high ICPI and MDT (see table I). Using this virus we obtain a plasmid containing the full length cDNA genome of NDV (fig. 1) and we make the modification in the amino acid sequence. After that, we rescue the recombinant virus which was called APMV-1/Mexico/RecP05/2010 (RecP05).As we describe in the methods section, the number of basic amino acids was changed to only two in the recombinant virus. With this change the ICPI and the MDT was lower as a lentogenic virus.

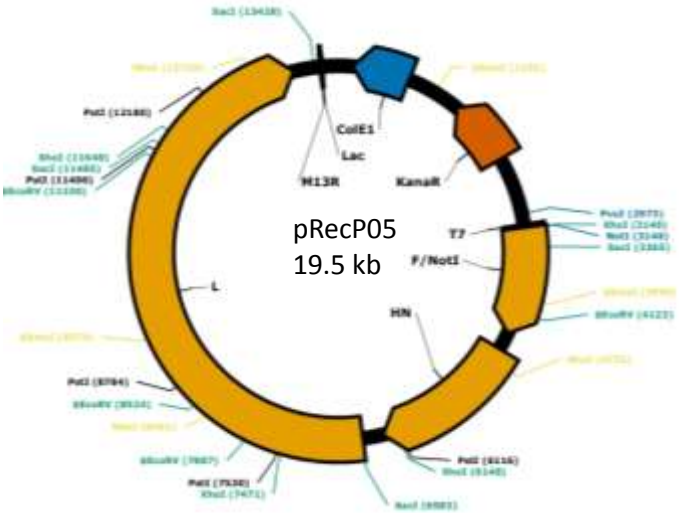

Fig.1 Map of plasmid pRecP05 containing the full length cDNA genome of NDV.

TABLE I. Virulence of the two virus

	P05	RecP05
ICPI	1.99	0.23
MDT	36 h	More than 96
AA Sequence	RRQKR	GRQGR

Conclusions. Changes in the amino acid sequence were sufficient to change the velogenic phenotype to a lentogenic one. This strategy can be used to develop vaccine seed from highly virulent virus.

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References.

1. Peeters B., et al. (1999). *J. of Virology*, 73 (6):5001-5009.
2. Absalón A. E. et al., (2012) Virus genes. 45:304–310
3. D.J. Alexander, in A Laboratory Manual for the Isolation and Identification of Avian Pathogens, (1989), p. 114