Molecular Characterisation of Three Avian Paramyxovirus Type 1 Isolated from Pigeons in France

CYRIL BARBEZANGE & VÉRONIQUE JESTIN*

AFSSA (French Agency for Food Safety), Avian and Rabbit Virology Immunology and Parasitology Unit,
BP 53, 22440 Plonéour-Lanvern Cedex, France

Received December 14, 2002; Accepted January 20, 2003

Abstract. Three avian Paramyxovirus type 1 (aPMV-1) isolated from pigeons during pigeon paramyxovirosis outbreaks were molecularly characterised by sequencing parts of the six genes (NP, P, M, F, HN and L) of each strain. Virulent 99143 isolate was found to be very closely related to non-pathogenic vaccine strains of aPMV-1, even for its F protein cleavage site motif. Strains 99299 and 99106, typical pigeon paramyxovirus type 1 (pPMV-1) variants, exhibited between 10% and 20% difference with aPMV-1 at the nucleotide level. The aPMV-1 specific pattern of eight amino acids in the intracellular domain of HN protein was found different by one residue for these two isolates, and might represent a specific pattern for pPMV-1. The unique sequence of the polycistronic P gene editing site of 99299 and 99106 was characterised by four instead of three cytosine residues, and might have an influence on the expression level of the three proteins encoded by P. This work is also the first to provide molecular data on NP, P and L genes of typical pPMV-1.

Key words: avian Paramyxovirus, fusion protein, hemagglutinin–neuraminidase, Newcastle disease virus, phosphoprotein, pigeon

Introduction

Virulent strains of avian paramyxovirus type 1 (aPMV-1 or NDV) are responsible for Newcastle disease, a highly contagious and economically important disease of poultry. Pigeon paramyxovirus type 1 (pPMV-1) are antigenic variants of aPMV-1 detected by several panels of monoclonal antibodies [1–5]. The pigeon disease was first described in the Middle East in the late 1970s [6] then reached Europe in 1981, and pPMV-1 strains were responsible for a panzootic in 1985, infecting racing and show pigeons [7]. Despite control measures, especially vaccination, pPMV-1 infection remains enzootic in pigeons in many countries [8]. The pPMV-1 isolates represent a threat for poultry productions: they were shown to be pathogenic for poultry both in experimental infections [9–11] and in natural outbreaks, as described in Great-Britain for chicken broilers, breeders and layers, in 1984 [12] and for pheasants in 1996 [13].

The aPMV-1, including pPMV-1, are members of the newly created genus *Avulavirus* within the viral family *Paramyxoviridae* (accepted proposal available on ICTV website at http://www.danforthcenter.org/ictv/ICTVnet) and contain a non-segmented single-stranded RNA genome of negative polarity. The approximately 15 kb RNA genome encodes the following six major polypeptides in the 3'-to-5' direction: nucleoprotein (NP), phosphoprotein (P), matrix protein (M), fusion protein (F), hemagglutinin–neuraminidase (HN) and RNA-directed RNA polymerase (L) [14]. The NP, P and L proteins together with the genomic RNA form the nucleocapsid involved in the transcription and the replication [15]. The M protein is involved in the budding process [16], and HN and F glycoproteins are anchored in the virion envelop and involved in the attachment and the fusion to host cells [17].
Molecular characterisation of aPMV-1 strains concerned mainly the F and M genes. The most studied molecular pattern was the F cleavage site which is considered as a major determinant of strain pathogenicity for poultry [18–20]. The nuclear localisation signal of M (between amino acid 246 and 263) has been targeted for epidemiological purposes [21]. However, particular fragments of other genes may be interesting. Thus, although the length of HN gene was associated with the potential pathogenicity of the strain [22], the sequence of the stalk might be also interesting as it was involved in the specific homologous cooperation with F protein [23]. The sequence around the transcriptional editing site of the polycistronic P gene (also coding for V and W proteins) seemed to be another interesting target for molecular characterisation [24,25]: the sequence of the editing site might influence the level of expression of each protein.

However, the only sequences available for pPMV-1 to date were restricted to the F cleavage site, the M nuclear localisation signal and the HN ending of a limited number of pPMV-1 strains [22,26,27]. In the present paper, to better characterise pPMV-1, we determined and analysed the partial sequence of the six genes for three avian paramyxoviruses type 1 isolated during pigeon paramyxovirus outbreak in France in 1999.

Material and Methods

Virus Strains

Following outbreaks that occurred in 1999, virus isolation was performed using embryonated chicken eggs (ECE) from conventional breeders, in a local veterinary diagnostic laboratory. Viral strain 99106 was isolated from a mix of lung, liver and intestine from a sacrificed racing pigeon, after having been passaged twice in ECE. Strain 99143 was similarly isolated from a mix of lungs and brains of unvaccinated racing pigeons from a loft where paralysis, anorexia and death were noticed and where pigeon sera were found positive for NDV IHA. Strain 99299 was isolated at the first passage in ECE, from a mix of brains from an unvaccinated pigeon loft where torticollis, greenish diarrhoea and death were noticed for both young and adult pigeons.

The antigenic characterisation of isolated haemagglutinating viruses was realised by haemagglutination inhibition test using specific antisera, following French COFRAC test Pr112/00/V/A30/00 [28], and reference monoclonal antibodies 3115,57,45b, specific for aPMV-1 [2], and 161/617, specific for pPMV-1 [1]. Their pathogenicity was evaluated by the intra-cerebral pathogenicity index (ICPI) performed into one-day-old specific-pathogen-free (SPF) chickens, according to European standards [29]. The aPMV-1 isolates 99106 and 99299 were assigned to the group P as described by Alexander et al. [30] since their haemagglutination property was inhibited by the specific monoclonal antibody 161/617. Based on this result and the value of their ICPI (0.9 and 1.4 respectively), they were characterised as “typical” pigeon variant PMV-1. Although the ICPI of 99143 isolate (1.4) was coherent with a mesogenic strain, its haemagglutination property was not inhibited by the monoclonal antibody 161/617. Complementary characterisation performed by the European Community Reference Laboratory at Weybridge, using the full panel of monoclonal antibodies described by Alexander et al. [30], confirmed the atypical characteristic of this isolate. There, it was then assigned to the group E (with avirulent vaccine strains as a B1-like strain), despite its virulent ICPI also confirmed in Weybridge (1.26).

RNA Extraction, Reverse Transcription and Polymerase Chain Reactions (RT–PCR)

The three selected virus strains were propagated once more in embryonated SPF chicken eggs to build up a working stock of infectious allantoic fluids. Viral RNA was extracted from 400 µl of infectious allantoic fluid with RNeasy® Mini kit (QIAGEN), according to the manufacturer’s instructions. Reverse transcription was realised with MMLV reverse transcriptase and hexamers.

All the PCRs were performed with Platinum® Taq DNA polymerase High Fidelity (Invitrogen), the mix containing 0.1 U/µl of polymerase, 0.05 mM each dNTP, 2 mM MgSO4 and 0.5 mM each primer. All the specific primers used in PCR and sequencing were designed with Oligo 4.1 software according to alignments of NDV strain sequences available in gene databanks and of preliminary sequencing results. Names and sequences of the different primer sets used in PCRs are available upon request. Two independent RT–PCR products were sequenced for each gene of each strain.