Short Communication

Genetic Characterization of Pigeon Paramyxovirus Type 1 in Egypt

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Abstract | This study reports the first pathotypic and genetic characterizations of the pigeon paramyxovirus type I (PPMV-1) from pigeon in Egypt during 2014. Pathotyping, based on the mean death time (86 hours) in eggs and the intracerebral pathogenicity index (1.2), and the amino acid motif, (^{112}KRQKR^{116}), at the cleavage site of the fusion (F) protein indicated the emergence of PPMV-1 in Egypt. Phylogenetic analysis of the F and haemagglutinin-neuraminidase (HN) genes indicated that the isolate clustered with the PPMV-1 strains recently reported from Israel within subgenotype VIb. Our findings report the emergence of PPMV-1 in Egyptian pigeons and the potential role these wild birds can play in the transmission and evolution dynamics of PPMV-1.

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Avian paramyxovirus serotype 1 (APMV-1), is a member of genus *Avulavirus* within the family *Paramyxoviridae*. APMV-1 comprises a diverse group of viruses with a single-stranded, negative-sense RNA genome of approximately 15.2 kb that encodes six structural proteins (Alexander and Senne, 2008). Three of them, the hemagglutinin-neuraminidase (HN), the fusion (F) and the matrix (M) proteins, are associated with the viral envelope. The remaining three proteins, the nucleoprotein (NP), the phosphoprotein (P) and the RNA polymerase (L) constitute the ribonucleoprotein complex (Aldous et al., 2003; Alexander and Senne, 2008).

NDV strains are classified into two major divisions based on the partial hypervariable region of the F gene, referred to as class I and class II. Class I has only one genotype (genotype 1) which further divided into three subgenotypes (1a, 1b and 1c). Class II is being divided into eighteen genotypes (I–XVIII) (Snoeck et al., 2013). Pigeon paramyxoviruses type 1 (PPMV-1), exclusively isolated from feral and domestic (racing or show) pigeons, is a host and antigenic variant of NDV/APMV-1 and predominantly represents distinct subgroup (subgenotype VIb) within genotypes VI of class II. Despite the fact that PPMV-1, primarily reported from the Middle East countries, were responsible for the third epizootic in the 1980s, the genetics and circulation patterns of PPMV-1 in these countries is still not completely understood (Diel et al., 2012; Guo et al., 2013).

In this study, a wild pigeon from Kafr El Sheikh province, Egypt was reported with clinical signs including

dullness, lethargy and severe neurological symptoms, which caused suspicion for PPMV-1 infection. Post mortem examination revealed the presence of petechial haemorrhages in the heart and brain, congested lungs and liver, and enlargement of spleen. Pooled homogenate of spleen and brain was used for isolation in 10-day-old specific-pathogen-free (SPF) embryonated chicken eggs (ECE) using standard procedures followed by haemagglutination-inhibition (HI) assay with polyclonal chicken antiserum against NDV (Alexander and Senne, 2008; WHO, 2012). To assess the pathogenicity of the viruses, mean death time (MDT) and intracerebral pathogenicity index (ICPI) were performed in ten-day-old embryonated eggs, and in one-day-old SPF chicks, respectively (Alexander and Senne, 2008; WHO, 2012).

RNA was extracted using TRIzol® Total RNA extraction kit from the allantoic fluid (Invitrogen, U.K) according to the manufacturer's recommendations. One-Step RT-PCR was performed using the Verso One-Step RT-PCR kit (Thermo, USA) according to the manufacturing instruction manual using specific primers for amplification of full-length F and HN genes (Munir et al., 2010). Sequencing of different fragments was achieved with Big-Dye terminator v 3.1 kit (Applied biosystems, USA) with both the forward and reverse primers to ensure a reliable consensus sequence. Multiple sequence alignments were performed using BioEdit software (Hall, 1999).

Phylogenetic analysis, based on the nucleotide sequences for the full-length F and HN genes of PPMV-1, was conducted using neighbour-joining method with 500 bootstrap replicates using MEGA5 program (Tamura et al., 2011). The sequences used in this study were submitted to the GenBank and are available under accession numbers; KU522142 and KU522143.

Recombination events in the full-length F (nt) and HN (nt) genes of PPMV-1 strain were examined using RDP v3.44 program (Martin et al., 2010). Seven different algorithms integrated in the program namely RDP, GeneConv, Chimaera, MaxChi, BootScan, SiScan and 3Seq were applied to detect the occurrence of any recombination events in PPMV-1 strain. Based on strain nucleotide sequences identity and the amino acid sequences of the full-length F and HN genes of Pigeon/Egypt/VRLCU/2014 isolate; a metathreading approach was applied in I-TASSER (Zhang and Skolnick, 2004; Zhang, 2008) to identify templates for the subjected sequences in a non-redundant protein database structure library. The structures were visualized and annotated in PyMol v1.3 software.

PPMV-1 strains can represent a significant threat to both domestic and wild bird populations; however, our understanding on the ecology, transmission dynamics and genetic diversity in this group of APMV-1 is extremely limited especially in Egypt. PPMV-1 strains are variants of NDV associated with infections of feral and domestic (racing or show) pigeons. These viruses likely originated from the Middle East and were responsible for the panzootic during the 1980s (Kaleta et al., 1985). In spite of control measures, PPMV-1 infection remained enzootic in many countries and caused a degree of economic lose (Guo et al., 2013).

In the present study, a PPMV-1 isolate collected from a pigeon was pathotypically and genotypically characterized. PPMV-1 was isolated from clinical samples collected from an infected pigeon through inoculation in specific pathogen free (SPF) eggs. Subsequently, the biological properties of the isolate, as determined by the MDT and ICPI, were calculated to be 86 hours and 1.2, respectively. Both these indices confirm the isolate, Pigeon/Egypt/VRLCU/2014, as pathogenic PPMV-1. Moreover, these observations were in line with the observed clinical outcome of the disease in pigeon.

Although the molecular basis of APMV-1 virulence relies on multiple genes, the difference in molecular determinants of APMV-1 virulence is mainly dependent on the amino acid sequence in the F protein cleavage site and the ability of specific cellular proteases to cleave the F proteins of different pathotypes (Aldous et al., 2003; Alexander and Senne, 2008). The amino acid sequence ^{112}-R/K-R-Q-R/K-R-F-^{117} is present in pathogenic AMPV-1, whereas the motif ^{112}-G/E-K/R-Q-G/E-R-L-^{117} is located in the cleavage site of lentogenic strains (Aldous et al., 2003; Alexander and Senne, 2008). The deduced amino acid sequence of the isolate under study revealed the presence of four basic arginine amino acids ^{112} R-R-Q-R-R^{116} motif with a phenylalanine residue at position 117 of the F protein (Figure 1). This motif is characteristics for the pathogenic strains of APMV-1 whereas the phenylalanine at this position has been suggested to be responsible for dissemination of the APMV-1 to nervous system and leads to neurological signs (Bulbule et al., 2015).

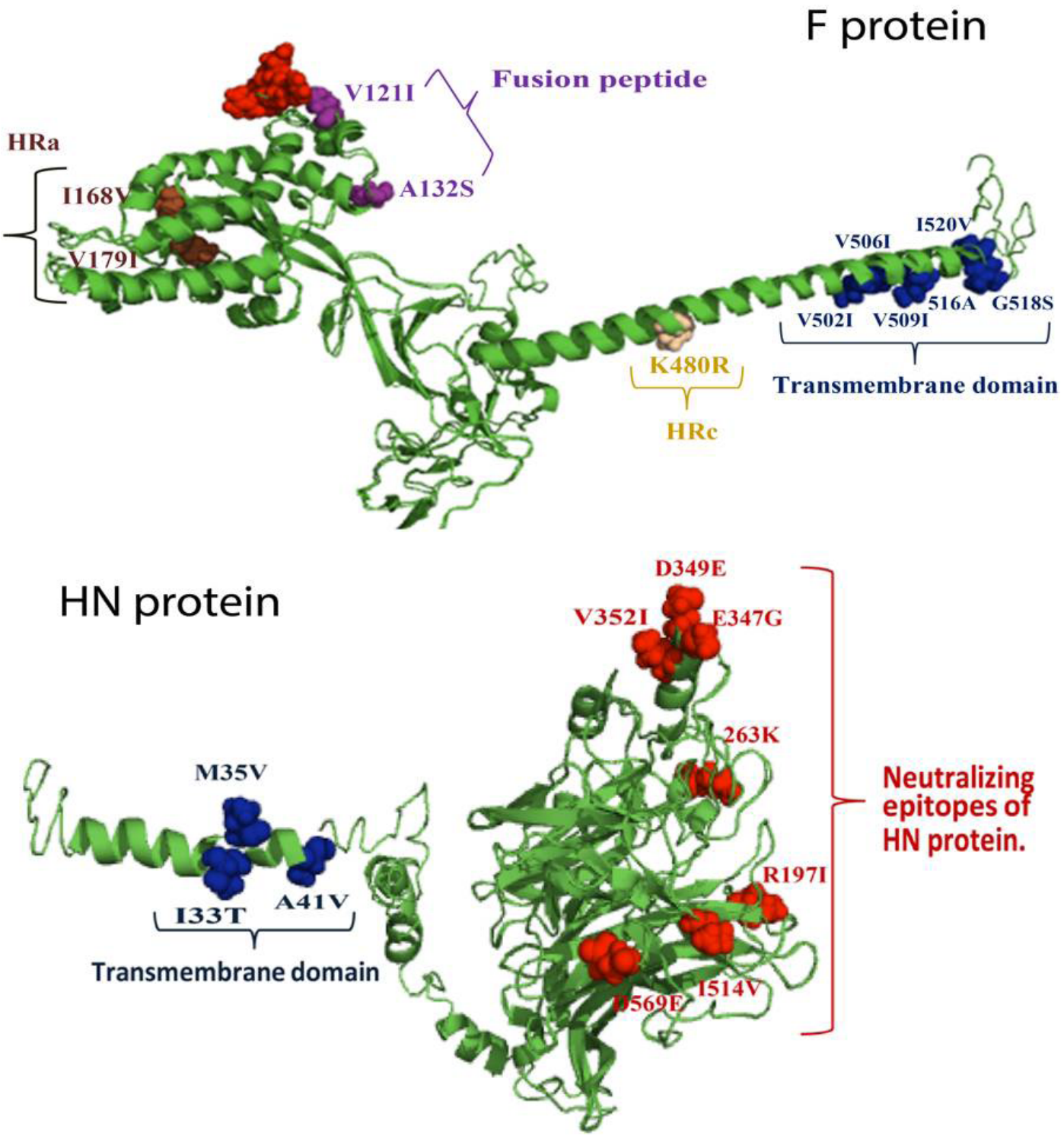

Figure 1: *Predicted 3D structures for F and HN proteins of PPMV-1 Pigeon/Egypt/VRLCU/2014 showing amino acids residues at cleavage site, fusion peptide, HR and transmembrane domain of F protein, and neutralizing epitopes and transmembrane domain of HN protein*

There were six potential glycosylation sites, Asn-X-Ser/Thr (N-X-S/T), in F protein, which were highly conserved in most NDVs. The major transmembrane region of isolate under study (501-521 aa) showed differences at position V502I, V506I, V509I, T/V/I/M516A, S518G and I520V (Figure 1). Compared with consensus amino acid sequences derived from NDV strains of different genotypes, the isolate under study had 2 substitutions (V121I, A132S) in fusion peptide (117-141 aa) (Figure 1). As reported, in the F protein, amino acid substitutions at fusion peptide and HR region, or replacement of transmembrane domain of NDV could affect the fusion activity of F protein (Alexander and Senne, 2008; WHO, 2012). Analysis of the three heptad repeat region (HR) showed 2 substitutions in HRa (143–185 aa) (V168I and V179I), and 1 in HRc (471–500 aa) (K480R) while there is no any substitutions at HRc (Figure 1).

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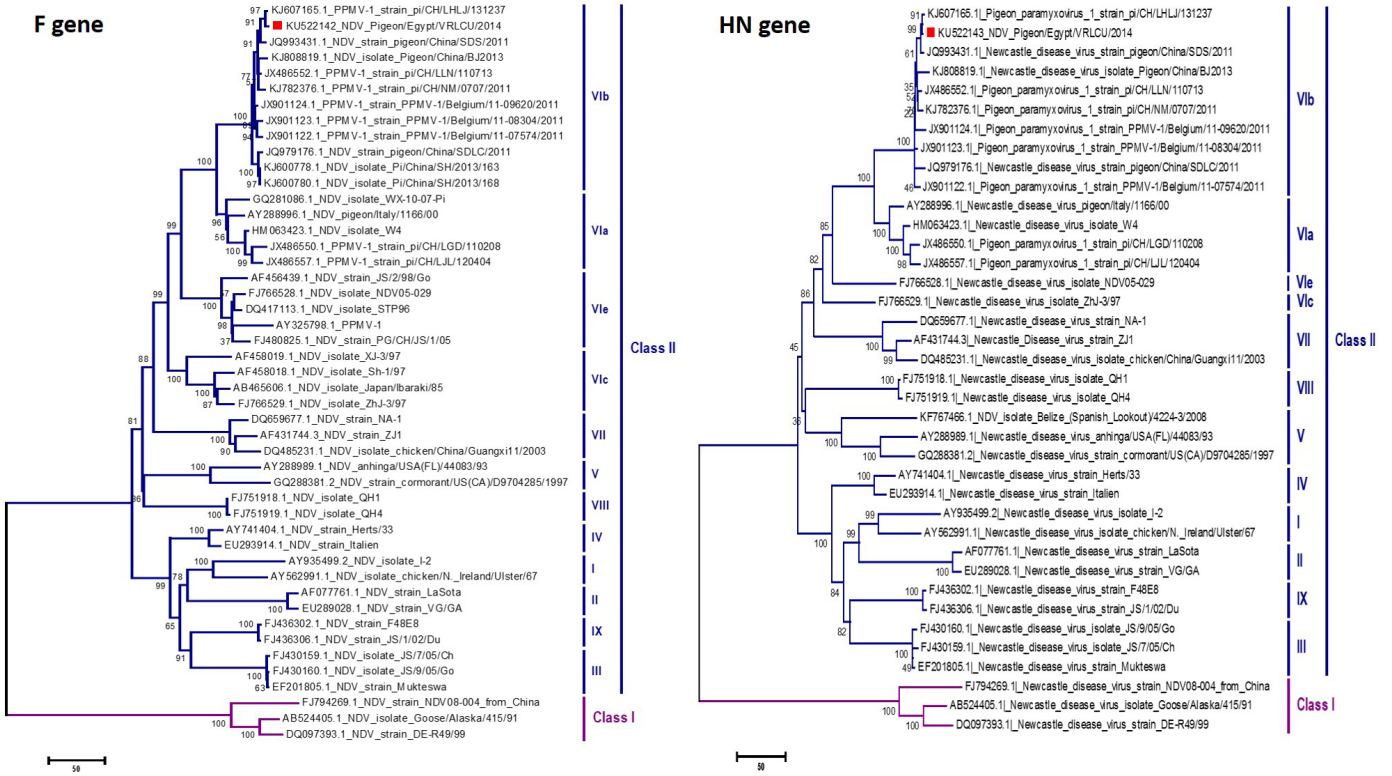

Figure 2: *Phylogenetic tree for F and HN genes of PPMV-1 Pigeon/Egypt/VRLCU/2014 Egyptian isolate based on nucleotide sequences showing the clustering within subgenotype VIb compared with other representative viruses of different NDV genotypes. The numbers at the nodes represent bootstrap values. Scale bar represents the number of substitutions per site. The year of isolation and geographical origin of the virus sequences are included in the tree. The reported isolate is marked with red filled square*

Moreover, there were 12 cysteine residues located at position 25, 27, 76, 199, 338, 347, 362, 370, 394, 399, 401, 424 and 523 of F protein.

The amino acids; 571 aa, 577 aa, 581 aa and 616 aa of HN protein was related to viral genotype (Hu et al., 2010). Some studies showed that the length of HN protein might contribute to virulence (Romer-Oberdorfer et al., 2006; Hu et al., 2010). In the transmembrane domain of the HN protein, there are 3 amino acid substitutions (I33T, M35V and A41V) (Figure 1). Our isolate under study had five potential glycosylation sites at position 119 (NNS), 341 (NNT), 433 (NKT), 481 (NHT) and 508 (NIS) of HN protein. Analysis of the neutralizing epitopes in the HN protein identified a total of four amino acid substitutions (R197I, N/S/Q263K, E347G, D349E, V352I, I514V and D569E) (Figure 1). Amino acid substitution in neutralizing epitopes was reported to play a role in formation of antigenic epitope and could result in neutralizing escape variants (Hu et al., 2010).

The virulence potential of genotype VI strains of PPMV1 in chickens is determined by virus strain, age of chickens, and the route of viral infections (Toro et al., 2005). However, the severity of infection by genotype VI (PPMV1) strains range from mild respiratory to neurotropic disease and mortality (Toro et al., 2005). Interestingly, serial passage of PPMV-1 in chickens or embryonated eggs can increase viral virulence, enhance the replication and might accompany induce mutations in two viral genes, P and L that have large effects on virus phenotype in different host species (Dortmans et al., 2011). Therefore, it is essential to closely monitor virulent PPMV-1, especially in birds in close proximity to poultry farms where the persistence of the PPMV-1 in chickens may lead to emergence of pathogenic APMV-1 strains.

A set of sequences, representing some reported genotypes around the globe, were aligned with the sequences presented in this study using the ClustalW algorithm in BioEdit (Hall, 1999). Phylogeny indicated that the isolate, Pigeon/Egypt/VRLCU/2014, clustered with the APMV-1 strains within genotype VI of class II (Figure 2). Genotype VI of APMV-1 has further been separated into either four (VIa–d) genotypes or six (VIa–f) sublineages (Diel et al., 2012). A phylogenetic analysis, by comparing the sequences within genotype VI, indicated that PPMV-1, Pigeon/Egypt/VRLCU/2014, was sub-categorized into sublineage VIb within class II based on the nucleotide

sequences for F and HN genes (Figure 2). Analysis of recombination in the PPMV-1 strain under study using RDP v3.44 program showed no recombination events.

In conclusion, we report here a pathogenic PPMV-1 belonging to subgenotype VIb isolated from pigeon in Egypt during 2014. Our characterization experiments have extended the biological information currently available on PPMV-1. Although the amount of PPMV-1 sequence data is growing, allowing the comparative sequence analysis necessary to evaluate transmission routes and origins of outbreaks, there remains a need for more full-length genomes. Therefore, the extensive surveillance of the wild birds for APMV-1, including its variant form, PPMV-1, is a fundamental requirement for understanding the epidemiology of these viruses.

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Conflict of Interests

The authors declare that they have no conflict of interest.

Authors' Contribution

M.A. Rohaim., R.F. El Naggar and N. Leblanc drafted and revised the manuscript. A.M. Helal and H.A. Hussein attended to the case and performed clinical sampling and follow-up. All authors contributed to the writing of the manuscript and approved it for submission.

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NOTE.1

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