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Molecular characterization of a pigeon paramyxovirus type 1 virus isolated from Eurasian collared doves in Iran, 2017

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ABSTRACT

In September 2017, an outbreak with high mortality, which showed the typical signs of ND, occurred among a flock of more than 2000 Eurasian collared doves in Konarak, southeast of Iran. A confirmed pigeon paramyxovirus type 1 strain was isolated from the brain tissues of the dead doves. The isolate, which was called Pigeon/Iran/Konarak/Barin/2017, was classified as a highly velogenic NDV. Complete genome sequencing and phylogenetic analysis showed that the isolate belonged to subgenotype XXI.2, which has never been reported from Iran before. The isolate had the highest homology (96.15%) with early 2010s Italian isolates. Further studies will be required to understand the diversity better.

Keywords: Pigeon paramyxovirus serotype-1 (PPMV-1); whole genome; fusion gene; phylogenetic study; subgenotype XXI.2; Iran

INTRODUCTION

NDV has a single-stranded negative-sense and non-segmented RNA genome [1]. The genome contains six genes of nucleocapsid (NP), phosphoprotein (P), fusion protein (F), matrix protein (M), hemagglutinin-neuraminidase (HN), and RNA large polymerase protein (L) that encode structural proteins with the same names. Although the HN and F glycoproteins are involved in the attachment and fusion of the virus to the host cell, the internal proteins are involved in several functions, such as transcription and replication of the virus [2,3].

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Conflict of Interest

The authors declare no conflict of interest.

NDV is divided into two classes (Class I and II) with one and 21 genotypes, respectively. According to the latest classification system, two new genotypes of XX, XXI, and some isolates of genotype VI, have been assigned to the NDV variant frequently reported from Columbiformes [4].

Although infections of pigeons by pigeon paramyxovirus type 1 (PPMV-1) have been reported since the 1930s, major events, such as the 3rd ND panzootic, are believed to have started during the late 1970s and have continued until now [5-8]. Indeed, potential transmission from Columbiformes to poultry has always been a concern in the poultry industry.

Doves are domesticated birds that come in close contact with backyard and commercial poultry [9]. They can also serve as reservoirs for different pathogens, such as ND [10]. In this study, the causative agent of an outbreak, i.e., PPMV-1, was characterized and analyzed phylogenetically in a flock of Eurasian collared doves. The PPMV-1 isolate belonged to subgenotype XXI.2, which has never been reported in the authors' geographical region.

MATERIALS AND METHODS

Case history

In September 2017, an outbreak with the typical symptoms of ND in a flock of more than 2000 wild Eurasian collared doves was reported in Konarak, Sistan, and Baluchestan southeast Iran (coordinates: 25°21′37″N 60°23′58″E). Brain tissue samples of dead doves were aseptically collected and stored at –70°C, which were used for virus isolation and identification According to the OIE protocols (OIE, 2018).

Pathogenicity study

The MDT test was conducted according to the OIE guidelines [11,12]. In addition, multiple candlings were used to determine the embryo time of death.

RNA extraction and RT-PCR

An RNX™-Plus Kit (CinnaGen, Tehran, Iran) was used for RNA extraction from the HI-confirmed allantoic fluids according to the manufacturer's instructions and stored at –70°C until needed. The cDNA was synthesized by applying of Revert Aid First Strand cDNA Synthesis Kit according to the manufacturer's instructions (Fermentas-Thermo Fisher Scientific, Canada). A partial fusion gene was amplified to confirm the presence of the NDV using primers and protocols described previously [13].

Genome sequencing

The primary results were confirmed by the generation of cDNA libraries for next-generation sequencing (NGS) on a HiSeq instrument (Illumina, USA) by BGI (China). The raw sequence data were analyzed and assembled within a customized workflow on the Galaxy platform, as described previously [14]. The sequences were assembled, edited, and analyzed using CLC sequence viewer 8 (CLC bio Anhus, Denmark).

Phylogenetic analyses

A phylogenetic tree for the F gene sequence was made using the standard pilot dataset prepared by the NDV consortium [4] (as of October 30, 2020). For HN and whole-genome comparisons, the sequences were selected based on the highest BLAST homologies and the originally

classified isolates in the NDV consortium datasets. The Maximum Likelihood method and a GTR+G model with a bootstrap of either 500 replicates were carried out using MEGA X [23].

RESULTS

An MDT of 52 h was obtained when the pathogenicity of the HI-positive isolate was examined. Preliminary PCR also showed that the isolate was the NDV. Amino acid (aa) sequence of the F protein cleavage site further showed that the virus contained a ^{112}RRQKRF^{117} motif, an indicator of a virulent strain. The isolate was called Avian_avulavirus_1_isolate_pigeon/Iran/Konarak/Barin/2017 (hereafter called Barin/2017), and its complete genome was deposited to GenBank under accession number MH044693.1. The size of the genome of the isolate was 15,192 bp.

According to BLAST analysis using the F gene sequence, Barin/2017 shared at most 96.15% identity with the PPMV-1 strains isolated from Israel and Italy in the early 2010s (namely MH377298.1 and N638234.1, respectively). Furthermore, Barin/2017 clustered to subgenotype XXI.2 (formerly subgenotype VIi under Diel et al. 2012 classification based on phylogenetic analysis of the F gene using the standard dataset of the NDV consortium [15]) (**Supplementary Data 1** and **Fig. 1**). Moreover, the genetic distance of the isolate was ~4% (below the 5% threshold) with other XXI.2 isolates, as shown in **Supplementary Data 2**.

BLAST and phylogenetic analyses based on the HN and the whole genome (15,192 b) were also conducted; similar results showing the same XXI.2 isolates were obtained.

DISCUSSION

NDV genotypes, such as VI, XX, and XXI, are sometimes referred to as PPMV-1 (shown in **Fig. 1** as a subtree derived from **Supplementary Data 1**), which are considered the most genetically diverse groups among the NDV strains [2]. Over the years, they have adapted to Columbiformes and became generally affiliated with them Moreover, sporadic outbreaks have been reported in poultry farms [16], making them an important issue that must be monitored and studied extensively.

In this study, the Barin/2017 isolate was categorized into subgenotype XXI.2, and is the first report of a subgenotype XXI.2 strain of an Iranian PPMV-1. Only two other complete genomes sequenced strains, belonging to subgenotype XXI.2, are currently available in GenBank, with the accession numbers of MH377298.1 and MG456676.1; the latter is an Iranian PPMV-1 and has some diversity in nucleotide sequences compared to the present isolate. Overall, Barin/2017 may belong to the subgenotype XXI.2 that has mutated since the early 2010s and was found in Iran or other parts of the world.

Only a few published reports on subgenotype XXI.2 are available. For example, in 2012, Bonfante et al. [17] reported three PPMV-1 strains classified into the subgenotype XXI.2 cluster (formerly VIi sub-genotype and sublineage 4a). Similarly, such viruses were isolated from collared doves during the surveillance carried out on wild birds in Italy in 2010–2011. In a different study, Snoeck et al. [18] reported two pigeon-derived subgenotype XXI.2 strains isolated from live markets in Nigeria. The analyses showed that Barin/2017 shared the same cleavage site motif of ^{112}RRQKRF^{117} with these subgenotype XXI.2 PPMV-1 isolates.

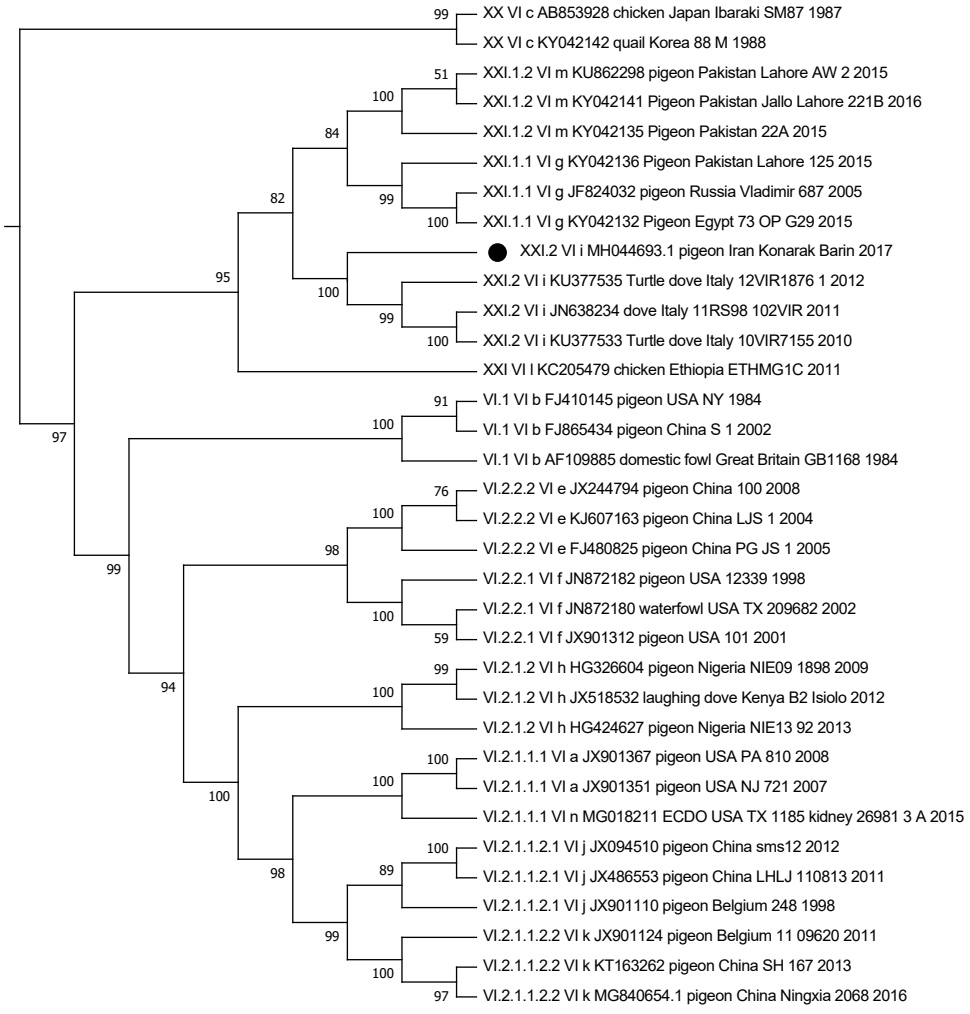

Fig. 1. Phylogenetic tree showing that Barin/2017 belonged to NDV subgenotype XXI.2 (marked with ●). The pigeon paramyxovirus type 1 subtree was derived from **Supplementary Data 1**, which was made using the F gene sequence of Barin/2017 and the standard pilot dataset of the NDV consortium [14].

Interestingly, the PPMV-1 subgenotype XXI.1.1 strains (previously called VIg) reported by other Iranian groups [19,20] did not show up more than 90% identity (the threshold set for the genotype and subgenotype proposed by the NDV consortium [4]) to Barin/2017, showing that other PPMV-1 genotypes/subgenotypes may also exist in Iran. Further study will be needed to understand the diversity of Iranian NDV originated from Columbiformes.

The subgenotype XXI.2 may be present and still circulating in other parts of the world, despite Barin/2017 being the last subgenotype XXI.2 reported.

This study provided the full genome sequence and characteristics of a subgenotype XXI.2 PPMV-1 strain, aiming to improve global NDV data. ND caused by PPMV-1 remains an ongoing panzootic since the 1980s, and it has become endemic in domestic and wild free-flying Columbiformes in most parts of the world. Therefore, more studies on this group of viruses will be needed because the circulation of PPMV-1 is a persistent threat to poultry farming in Iran and other countries.

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SUPPLEMENTARY MATERIALS

Supplementary Data 1

Evolutionary analysis inferred using the Maximum Likelihood method and General Time Reversible model [21] using the standard pilot dataset of the NDV consortium [4] and the F gene sequence of Barin/2017. The isolate clustered to the subgenotype XII.2 strains. The bootstrap consensus tree inferred from 500 replicates [22] was taken to represent the evolutionary history of the taxa analyzed. A discrete Gamma distribution was used to model the evolutionary rate differences among the sites (four categories [+G, parameter = 0.4095]). All positions containing gaps and missing data were eliminated (complete deletion option). A total of 1662 positions were in the final dataset. Evolutionary analyses were conducted in MEGA X [23]. Barin/2017 was marked with ●.

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Supplementary Data 2

The evolutionary distance based on the sequence of the F gene. Barin/2017 showed ~4% distance (below the 5% threshold) to the subgenotype XXI.2 strains in the standard dataset of the NDV consortium, suggesting it belonged to the same cluster. The numbers of base substitutions per site from between the sequences are shown. Standard error estimate(s) are shown above the diagonal. The analyses were conducted using the Maximum Composite Likelihood model [24]. All positions containing gaps and missing data were eliminated (complete deletion option). There were 1662 positions in the final dataset. Evolutionary analyses were conducted in MEGA X [23].

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