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PIGEON PARAMYXOVIRUS IN WILD AVIFAUNA OF KAZAKHSTAN

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ABSTRACT

Field materials such as cloacal and tracheal swabs were collected from aquatic and terrestrial birds at the ornithological station Shakpak (Jambyl region) in 2014 and 2016. Twenty-three samples were obtained from two species of Columbiformes: the rock dove and the rufous turtle dove. Positive samples were found in eleven of the twelve samples obtained from the rock dove, while samples from the rufous turtle dove were negative. The article describes the comparative molecular-genetic and phylogenetic properties of velogenic Newcastle disease virus (NDV) strains isolated in southern Kazakhstan. To determine the pathotype of the Kazakhstan isolates from wild birds, the gene encoding the fusion protein was sequenced. The proteolytic cleavage site was defined as RRQKR*F, which corresponds to a velogenic/mesogenic serotype. The partial F gene-based sequence analysis revealed an evolutionary relationship to sub-genotype VIb formed by strains isolated in geographically close regions — south of western Siberia and Altai, and also to a virus that had previously circulated in southeast Kazakhstan. Investigations conducted in this field allow for the identification of regions in Kazakhstan that are susceptible to NDV outbreaks. The findings will also enhance our knowledge of circulating PPMV strains in Kazakhstan. Extensive environmental and epizootological studies of PPMV are of particular importance for the safety of local poultry farming.

Keywords: Pigeon pavamyxovirus, Newcastle disease virus, avian avulavirus, dove, sequencing, genotype VIg

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INTRODUCTION

Avian paramyxoviruses (APMV) are viruses with negatively charged linear RNA circulating among humans, animals, birds and reptiles. The viral RNA is synthesized and translated in the host cell, and is represented by six or seven genes encoding three envelope proteins: matrix (M), fusion protein (F) and hemagglutinin-neuraminidase (HN), as well as three core polypeptides - nucleoprotein (NP), phosphoprotein (P) and large protein (L). APMV of serotype 6 has an additional gene coding the small hydrophobic protein (SH), which is absent in other APMV. The functional role of the envelope proteins is to ensure the attachment and penetration of the virus into the infected cell, while the internal polypeptides are involved in viral RNA replication [1].

APMV or, according to the new classification, Avian avulavirus include twenty serotypes, affecting many wild and domestic bird species worldwide [2]. The most common among all Avian avulaviruses is Newcastle Disease Virus (NDV), referring to Avian avulavirus 1 (AAvV-1). For the first time, NDV was isolated from poultry in Indonesia on the island of Java in 1926 [3], and in 1927 an epizootic was observed in a chicken farm near the city of Newcastle (England), from which the disease later got its name. Based on mean death time in chicken embryos, NDV strains are divided into three pathotypes: velogenic (highly pathogenic), mesogenic (moderately pathogenic) and lentogenic mostly with asymptomatic manifestations [4].

According to recent new classification, all NDV consist of two highly divergent classes 1 and 2 that further divided into genotypes. A ten per cent mean nucleotide difference of the fusion protein gene coding sequence is necessary to assign phylogenetically distinct groups of AAvV-1 into genotypes. Class 1 isolates belong to a single genotype whereas class 2 isolates are divided into 18 genotypes. Some genotypes are further divided into sub-genotypes based upon calculations of intra-genotype genetic diversity [5].

Strains of class 1 were isolated from both domestic and wild birds in North America and Eurasia since 1970s [6]. Members of this class constitute a single phylogenetic group, designated as genotype 1. Further analysis showed that within genotype 1, there were three sub-genotypes 1a, 1b and 1c.

To date there are XVIII genotypes identified within the Class 2. These viruses were isolated from a large number of wild and domestic birds, the predominant part of them is velogenic and causes huge economic loss to poultry industry [7].

NDV isolated from pigeons since the 1980s were assigned to the genotype VI of class 2 and were called “pigeon paramyxoviruses”(PPMV). The first outbreaks of this disease in pigeons were recorded in the late 1970s and early 1980s in Eurasia, which then developed into a panzootic, which continues to date [8]. Pigeon paramyxoviruses from doves and pigeons are considered as virulent variants of avian avulavirus 1 that are adapted to those species and spillover into domestic poultry have been regularly reported worldwide [9, 10, 11, 12, 13].

This article describes a case of isolating velogenic NDV strains from rock dove in Southern Kazakhstan, their comparative molecular-genetic and phylogenetic properties. Due to the widespread of NDV in nature and the enormous economic damage caused to poultry farms, extensive environmental and epizootological studies of NDV in various regions of the world, including Kazakhstan, are of particular importance.

Materials and methods

Field materials as cloacal and tracheal swabs were collected from aquatic and terrestrial birds belonging to various species as well as from poultry according to Office International des Epizooties (OIE) approved techniques [14] and stored in a liquid nitrogen (-196°C) until delivery to the laboratory.

Isolation of the virus and recovery passages were conducted by inoculation of each sample into the allantoic cavity of 9-10 days old embryonated chicken eggs with subsequent incubation at 36°C for 48 hours according to certified methods recommended by the OIE.

Viral RNA was extracted using QIAamp Viral RNA Mini Kit (Qiagen, Hilden, Germany) according to the manufacturer's recommendations.

RT-PCR was conducted using OneTaq One-Step RT-PCR Kit (NEB, USA) in Eppendorf Gradient thermocycler with primers targeting the fragment of the F-gene [7].

For Sanger sequencing, gel with bands was excised and purified using Quick Gel Extraction Kit (Invitrogen, Germany) and sequenced on an ABI 3500 DNA analyzer

(Applied Biosystems, USA) using BigDye Terminator v.3.1 Sequencing Kit (Applied Biosystems, USA).

Alignment of the NDV F-gene sequences was carried out in MEGA7.0 software. Phylogenetic analyses were carried out by neighbor joining method applying the Tamura 3-parameter model [15] with 1000 bootstrap replications to assign confidence levels to branches also in MEGA7.0 [16].

RESULTS AND DISCUSSION

During the collection of field material at the ornithological station Shakpak (Jambyl region) in 2014 and 2016, twenty three samples were obtained from 2 species of Columbiformes: Rock Dove and the Rufous Turtle Dove (Table 1).

Table 1. List of collected samples from wild birds in Jambyl region

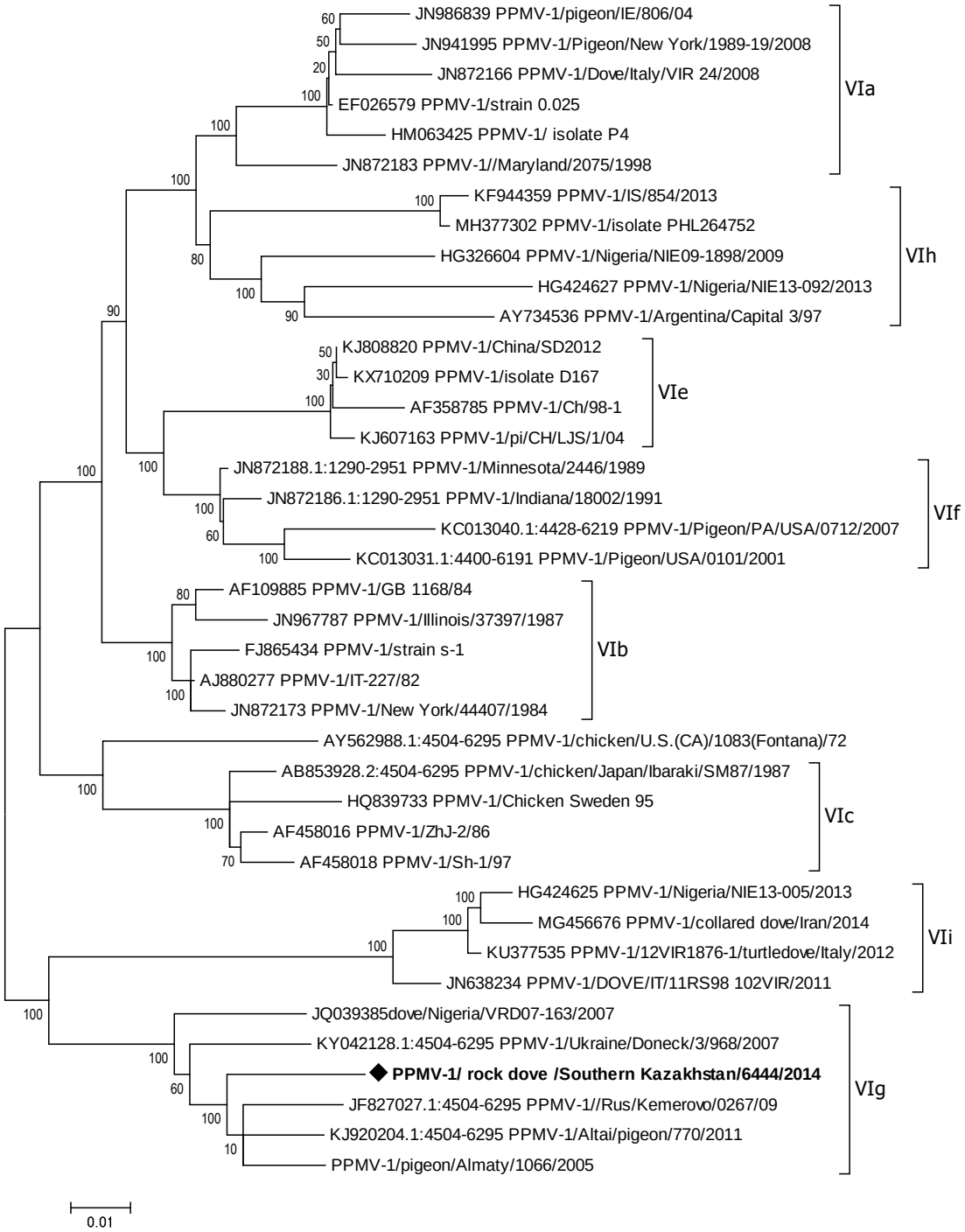
Order	Species (Latin Name)	Species (English Name)	Number of samples collected in	
			2014	2016
Columbiformes	*Columba livia*	Rock Dove	12	-
Columbiformes	*Streptopelia orientlis*	Rufous Turtle Dove	1	10
Total	2	-	13	10

Chicken embryos were infected with bird samples, followed by incubation at 36 ° C for 48-72 hours. The presence of hemagglutinating agents (HAA) was checked with 0.75% chicken erythrocytes. As a result of the initial infection of chicken embryos and subsequent passages, HAA were obtained.

The screening of the isolated HAA in RT-PCR was carried out with primers targeting the fragment of the F gene, which cover the genome region of 679 b.p. Positive samples were found in eleven of the twelve samples obtained from rock dove, while samples from rufous turtle dove were negative. Also, in the course of research of materials from the Zhambyl region, additional three isolates of pigeon paramyxoviruses from birds of other orders *Passeriformes* and *Accipitriformes* were identified. According to the international nomenclature, the identified isolates were called:
AAvV-1/ rock dove /Southern Kazakhstan/6438/2014;
AAvV-1/ rock dove /Southern Kazakhstan/6439/2014;
AAvV-1/ rock dove /Southern Kazakhstan/6440/2014;
AAvV-1/ rock dove /Southern Kazakhstan/6441/2014;
AAvV-1/ rock dove /Southern Kazakhstan/6442/2014;
AAvV-1/ rock dove /Southern Kazakhstan/6443/2014;
AAvV-1/ rock dove /Southern Kazakhstan/6444/2014;
AAvV-1/ rock dove /Southern Kazakhstan/6445/2014;
AAvV-1/ rock dove /Southern Kazakhstan/6446/2014;
AAvV-1/ rock dove /Southern Kazakhstan/6447/2014;
AAvV-1/ rock dove /Southern Kazakhstan/6448/2014;
AAvV-1/myna/Southern Kazakhstan/6434/2014;
AAvV-1/ long-legged buzzard /Southern Kazakhstan/6449/2014;
AAvVV-1/ golden eagle /Southern Kazakhstan/6451/2014.

To determine the pathotype of the Kazakhstan isolates from wild birds, sequencing of the gene encoding the fusion protein was performed. The putative amino acid sequence of the cleavage site of this protein consisted of the basic amino acids KRQRR*F in positions 112-116, which is a sign of the high pathogenicity of viruses. All the obtained sequences were identical to each other, so only one isolate was taken for further analyses.

To study the evolutionary relationships of the Kazakhstan strain with viruses previously circulated Kazakhstan and in the world, a phylogenetic tree was built on the basis of the F-gene sequence using the MEGA 7.0 software. The results are shown in Figure 1.



Note - The Kazakhstan isolate is highlighted with a filled diamond before the isolate name.

Fig. 1. Phylogenetic relationships for the F-gene of the Kazakhstan PPMV isolate with those from the Genbank database

As can be seen from the phylogram on Figure 1, the tree is formed with pigeon paramyxoviruses isolated in different parts of the world and belong to the class 2 within genotype VI. The genotype is divided into eight VIa-VIi sub-genotypes (the VId

genotype is absent). The Kazakhstan isolate PPMV-1 / rock dove / Southern Kazakhstan / 6444/2014 was phylogenetically close to strains belonging to the sub-genotype VIg, isolated in geographically close regions — south of Western Siberia and Altai, and also to a virus that had previously circulated in Southeast Kazakhstan.

Birds of the *Columbidae* family are the main reservoir of highly virulent pigeon paramyxoviruses of genotype VI in nature. Historically, large die-offs in doves and pigeons were reported in the Almaty region in Kazakhstan in 2005 [9] and the isolate under study was phylogenetically close to those viruses. Mainly, all viruses within class 2 genotype VI are considered as virulent in poultry. Viruses belonging to the sub-genotype VIg have been isolated from pigeons and a doves in Nigeria, Russia, and Ukraine during 2005–2011 [17, 18, 19]. The similarity of the isolate under stidy to them and its regular isolation in Central Asia indicates a constant circulation in the region.

In addition to the danger of mechanical introduction of the velogenic strain to poultry farms with subsequent mass die-offs, another threat is the formation of a pigeon paramyxovirus lineage adapted to domestic birds, causing regular lethal outbreaks, as happened in Ethiopia in 2011-2012 [20].

Investigations conducted in this field, allow identifying regions in Kazakhstan susceptible to NDV outbreaks. Identification of areas with the highest incidence of epizootics will contribute to rapid response, localization and elimination of the outbreak consequences. Annual monitoring of the circulating NDV strains is urgent for practical epidemiology, since its results can be used in identifying outbreaks of disease and carrying out the necessary preventive measures.

CONCLUSION

1. During the virological study of samples from wild pigeons collected in the southern regions of Kazakhstan in 2012-2014, thirteen hemagglutinating agents identified later as pigeon paramyxoviruses were identified.
2. Sequencing of the fragment of the F-gene has shown the presence of the putative basic amino acids KRQRR*F, which is a sign of the high pathogenicity of viruses.
3. It was established that Kazakhstan strains belong to the group of pigeon paramyxoviruses of VIg sub-genotype, which caused lethal outbreaks among pigeons in Africa and Eurasia.
4. The continuous circulation of viruses of this genotype among pigeons in Central Asia, pose a potential threat to poultry farms in the region.

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