Co-circulation of genetically distinct groups of avian paramyxovirus type 1 in pigeon Newcastle disease in Iran

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ABSTRACT
Pigeons are considered as one of the major natural reservoirs in the epidemiology of Newcastle disease (ND). In this study, the partial sequence of fusion protein gene of 17 pigeon-origin ND viruses (NDVs) isolated during 2012–2013 in Iran was analysed. Since the studied isolates showed F0 protein cleavage sites compatible with velogenic NDVs, all were considered as virulent NDVs. Two isolates carried 112RRQKRF117 as the cleavage site motif, whereas the rest demonstrated 112KRQKRF117 motif which just recently has been reported among Iranian virulent NDVs. Phylogenetic analysis divided all these diverse isolates in two distinct clusters within class II genotype VI. Based on the partial fusion protein gene sequence, 15 out of 17 isolates showed the highest genetic identity to subgenotype VIb/2 and the other two isolates were placed in a distinct genetic group of genotype VI. Based on recent findings, at least two different sublineages of genotype VI are causing the ND outbreaks in the pigeon population and are circulating simultaneously along with virulent NDVs of genotype VII in various species in Iran. The continuing circulation of a diverse group of virulent NDVs as an enzootic in widespread species such as pigeon can cause outbreaks in commercial poultry flocks and also failure in controlling programmes. Therefore, the constant monitoring and awareness of the virus characteristics should be considered in controlling programmes against ND in Iran.

Introduction
Newcastle disease (ND) is a detrimental worldwide disease having had significant impact not only on the poultry industry but also on the world economy. This economical effect is due to the enzootic or sporadic outbreaks in birds, subsequent trade restrictions and the cost of control programmes (Alexander et al., 2012).

Newcastle disease viruses (NDVs) are a diverse group of viruses that all belong to serotype 1 of avian paramyxovirus (APMV-1) along with 12 other serotypes constitute genus of Avulavirus of the Paramyxoviridae family (Alexander et al., 2012; Terregino et al., 2013; Yamamoto et al., 2015).

NDV isolates have been characterized based on pathogenicity, antigenicity, the genome size and the nucleotide sequence of the fusion protein (F) gene (Alexander et al., 1985; Collins et al., 1993; Ujvári et al., 2006; Diel et al., 2012). Different lineages or genotypes systems based on the phylogenetic analysis of the partial or complete nucleotide sequences of the F gene have been developed to classify NDV isolates (Aldous et al., 2004; Miller et al., 2010). The genotypes system, which is the mostly used system, classifies NDVs in two major groups of class I and II (Miller et al., 2010). However, both systems have led to much confusion in NDVs classification. Diel et al. (2012) attempted to prepare a unified nomenclature and classification system of NDV genotypes based on the phylogenetic analyses of all complete F gene sequences available on GenBank. They proposed that class I viruses contain only a single genotype, whereas class II NDV isolates are divided into 18 genotypes. However, the suggestion of novel genetic group is still occurring (Courtney et al., 2013; De Almeida et al., 2013; Snoeck et al., 2013).

The third panzootic of ND was caused by variant strains of NDV, designated as pigeon paramyxovirus type 1 (PPMV-1) (Alexander et al., 1985; Aldous et al., 2004). Despite the initial occurrence in pigeon flocks, multiple outbreaks of PPMV-1 have been reported in chickens since the early 1980s and this situation remains a perpetual threat to the poultry industry (Alexander, 2011; Aldous et al., 2014).

Having been reported in multiple species such as wild birds, various domestic poultry and as frequent outbreaks in commercial chicken flocks since the first confirmation in 1951, ND is presently considered to be enzootic in Iran (Sohrab, 1974; Bozorgmehri-fard & Keyvanfar, 1979; Momayez et al., 2007; Ghiamirad et al., 2010; Madadgar et al., 2013; Hosseini et al., 2014). In the ND control programmes in enzootic regions, constant monitoring and identification of the causative agent are required (Miller et al., 2013). Yet,