



## RESEARCH ARTICLE

# Identification of Newcastle Disease Virus sub-genotype VII 1.1 isolated from chickens in Sabah, Malaysia

Syamsiah Aini, S.<sup>1\*</sup>, Leow, B.L.<sup>1</sup>, Faizul Fikri, M.Y.<sup>1</sup>, Muhammad Redzwan, S.<sup>1</sup>, Faizah Hanim, M.S.<sup>1</sup>

<sup>1</sup>Veterinary Research Institute, Ipoh, Perak, Malaysia

\*Corresponding author: syamsiah@dvs.gov.my, syamsi\_aini@yahoo.com

### ARTICLE HISTORY

Received: 30 December 2021  
Revised: 28 November 2022  
Accepted: 30 November 2022  
Published: 31 December 2022

### ABSTRACT

Newcastle disease (ND) is an extremely contagious and fatal viral disease causing huge economic losses to the poultry industry. Following recent ND outbreaks in Sabah in commercial poultry and backyard farms, it was speculated that this could be due to a new introduction of Newcastle Disease Virus (NDV) genotype/sub-genotype. Here we report the genetic characterization of NDVs isolated from Sabah during early 2021. All isolates were amplified and sequenced with primers specific to the viral fusion (F) gene using reverse transcription-polymerase chain reaction (RT-PCR). Nucleotide sequence analysis of the F gene showed that all isolates shared similar homology of 99.4% with NDV strain from Iran isolated in 2018. Amino acid sequences of the F protein cleavage site revealed the motif of <sup>112</sup>RRQKRF<sup>117</sup> indicating all isolates were of virulent strain. Phylogenetic analysis demonstrated that all isolates were clustered under sub-genotype VII 1.1 and clustered together with isolates from Iran (previously known as sub-genotype VIII). The present findings suggested that there is an emerging of a new sub-genotype into the poultry population in Sabah and this sub-genotype has never been reported before in Malaysia. Therefore, transboundary monitoring and continuous surveillance should be implemented for proper control and prevention of the disease. A further molecular epidemiological analysis of NDV is needed to well understand the circulatory patterns of virulent strains of NDV in the country to prevent future outbreaks.

**Keywords:** Newcastle Disease Virus (NDV); VII.1.1; chickens; Sabah; Malaysia.

### INTRODUCTION

Newcastle disease (ND) is a contagious viral disease that affects various avian species, mainly domestic poultry and causes major outbreaks (Ghalyanchilangeroudi *et al.*, 2018). ND is caused by virulent strains of *Avian Orthoavulavirus serotype 1 (AOAV-1)* viruses or generally referred to as Newcastle Disease Virus (NDV) (Amarasinghe *et al.*, 2018; ICTV, 2019). The NDV has a negative-sense RNA genome of 15kb, with six genes encoding structural fusion (F), haemagglutinin-neuraminidase (HN), nucleocapsids (N), phosphoprotein (P), matrix (M), and large polymerase (L) proteins (Hussain *et al.*, 2020; Nagy *et al.*, 2020). Additionally, two non-structural proteins known as V and W are produced as a result of RNA editing of the P gene (Ganar *et al.*, 2014).

Among the proteins, the F and HN proteins are surface glycoproteins and protective antigens of NDV (Ghalyanchilangeroudi *et al.*, 2018). The F protein directs the viral fusion activity whereas the HN protein is responsible for virus attachment (Peeters & Koch, 2021). Intracerebral pathogenicity index (ICPI) can be used to determine NDV pathotype. However, ICPI should only be used where there is a strong epidemiological justification due to the severity of the procedure (OIE, 2021). Alternatively, the virulence of the virus can be determined by analysing the amino acid sequence of the F0 protein cleavage site (position 112–117) (Wang *et al.*, 2017).

According to the updated classification by Dimitrov *et al.* (2019), complete F gene nucleotide sequencing classified NDVs into two major classes: class I and class II. Viruses from Class I belong to a single genotype, while for Class II viruses, 21 genotypes (made up of several sub-genotypes) have been identified. Avirulent isolates from wild waterfowl are mostly found in Class I (Dimitrov *et al.*, 2019). The majority of NDV strains are classified as Class II, which includes both virulent and avirulent strains (Alexander *et al.*, 2012). The genotype VII landscape has changed as a result of the new classification. Genotype VII was reclassified into only three sub-genotypes: VII.1.1, VII.1.2 and VII.2. Sub-genotypes VIIb, VIId, VIIe, VIIj, and VIII were combined into single sub-genotype VII.1.1, VII.1.2 included sub-genotype VIIf and VII.2 included the sub-genotypes (h, i and k) (Dimitrov *et al.*, 2019).

In Malaysia, the first ND outbreak was reported in Parit Buntar, Perak, in poultry flocks in 1934 (Mahamud *et al.*, 2021). Since then, ND is considered endemic as it had caused major outbreaks not only in vaccinated chickens but also village chickens and non-poultry avian species (Shohaimi *et al.*, 2015). Six genotypes (I, II, III, VI, VII and VIII) have been reported to circulate in Malaysia (Shohaimi *et al.*, 2015; Mahamud *et al.*, 2021). For the past 20 years, NDV genotype VII has been the predominant genotype in Malaysia (Tan *et al.*, 2010; Berhanu *et al.*, 2010; Shohaimi *et al.*, 2015; Aljumaili *et al.*, 2017; Syamsiah *et al.*, 2021).

In early 2021, reports of high mortalities up to 50% with respiratory and enteric clinical signs in many areas in Sabah were received not only in commercial farms but also village chickens by Sabah Department of Veterinary Services. The authority conducted disease investigations and sample collection. Virus isolation was carried out by the Veterinary Diagnostic Laboratory Kota Kinabalu, Sabah. Allantoic fluid was harvested and tested positive for NDV by Real Time RT-PCR. The allantoic fluids were then sent to Veterinary Research Institute (VRI) Ipoh for ND confirmation and genotyping. In a recent study by Syamsiah *et al.* (2021), it was reported that NDV was detected in Sabah in 2019 from *ayam kampung* and fighting cock. Even though it is from genotype VII, but the isolates do not cause major outbreaks in Sabah. This study was, therefore conducted to determine the genotype of the NDV from the recent outbreaks to have a clearer understanding of NDV circulation in Sabah.

## MATERIALS AND METHODS

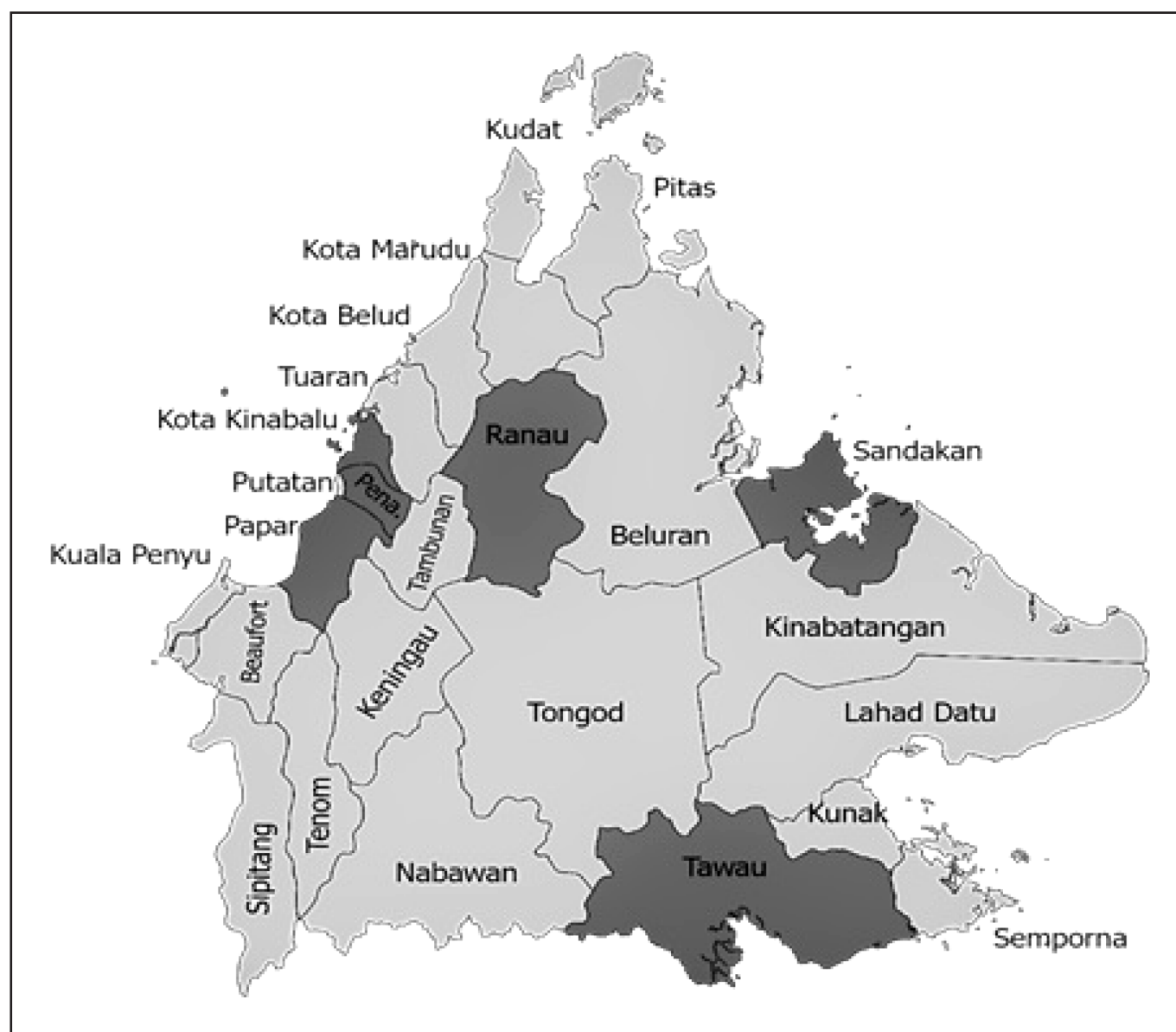
### Virus Isolation

During January and February 2021, a total of 20 allantoic fluid samples from Veterinary Diagnostic Laboratory Kota Kinabalu, Sabah were sent to Avian Virology Section, VRI, Ipoh for confirmation and

genotyping of NDV. The samples were propagated in 9 to 11 days old Specific Pathogen Free (SPF) embryonated chicken eggs via the intra-allantoic route and incubated for three days at 37°C. The allantoic fluids were harvested and tested for hemagglutination activity via haemagglutination (HA) test according to OIE (2021). The positive HA isolates were examined later by haemagglutination inhibition (HI) test using specific antisera against ND (OIE, 2021).

### Extraction of viral Ribonucleic acid (RNA) and real-time reverse transcription - polymerase chain reaction (qRT-PCR)

All 20 allantoic fluid samples received from Sabah were also tested for molecular detection for ND. The samples were first screened for ND by one-step Taqman real-time RT-PCR (qRT-PCR) assay using primers and probe specific for NDV (matrix gene) (Wise *et al.*, 2004). The presence of virulent F gene in positive isolates was investigated by qRT-PCR test, according to the method suggested by Wise *et al.* (2004). The real-time assays were performed in the QuantStudio 3 real-time PCR system (Applied Biosystems, USA). Out of the 20 isolates, six isolates were taken for further molecular characterization of full length of F gene of the NDV. These six isolates were representative from different parts of Sabah; the Papar, Kota Kinabalu, Ranau, Penampang, Sandakan and Tawau (Figure 1).



**Figure 1.** Map of Sabah showing the area of NDV positive cases in during January and February 2021. Maps were created using paintmaps.com. Areas affected are shaded dark grey.

**Amplification and Sequencing of Fusion gene**

Total viral RNA was extracted from 200 µl of infected allantoic fluid using Indispin Pathogen kit (Indical Bioscience, Germany) following the manufacturer’s instructions. One step RT-PCR was carried out using the SuperScript III One-Step RT-PCR System with Platinum Taq (Invitrogen, USA). Three sets of primers used in the amplification of complete F gene are shown in Table 1. A PCR program was set in the T100 Thermal Cycler (Bio-Rad, USA) for amplification of templates which included RT at 48°C for 30 min, initial denaturation at 94°C for 5 min, followed by 35 cycles of denaturation at 94°C for 1 min, annealing at 50°C for 1 min and extension at 68°C for 2 min with a final extension for 10 min at 68°C. The amplicons were then analysed by gel electrophoresis using 1.0% agarose gel stained with SYBR Safe DNA gel stain (Invitrogen, USA). The amplified PCR products were cut from the gel and sent for Sanger sequencing (Apical Scientifics (M) Sdn Bhd). The primers used in DNA sequencing are the same as those used in RT-PCR amplification.

**Sequence and Phylogenetic Analysis**

The raw sequences were manually edited and assembled using SeqMan Pro software (DNASTar Lasergene, USA). The alignment and comparison of the sequences in this study and other published sequences was performed by the BioEdit Sequence Alignment Editor version 7.1.9 (Hall, 1999). The sequences were assessed using Basic Local Alignment Search Tool (BLAST) and compared to other sequences in the GenBank NCBI (NCBI, 2016).

Phylogenetic analysis was conducted, and evolutionary distance was analysed using MEGA version 7.0.26 by using the maximum likelihood (ML) method with Kimura 2-parameter model and setting bootstrap 1000 replicates (Tamura *et al.*, 2013). The phylogenetic tree was generated based on the complete F gene from nucleotide 1 to 1662. As recommended by the recent NDV classification system by Dimitrov *et al.* (2019), distances of more than 5–10% and 10% were used to assign sub-genotypes and genotypes respectively.

Sequences generated in this study were submitted to the GenBank database to obtain the accession number and their accession numbers are OK338510, OK338511, OK338512, OK338513, OK338514 and OK338515.

**RESULTS**

**Virus Isolation**

All of the Sabah samples were successfully isolated in SPF embryonated eggs. The harvested allantoic fluid samples were HA

positive, with HA titres ranging from 64 to 256. The viruses were neutralised by antiserum specific to ND in the HI assay, with the HI titre ranging from 32 to 128 indicating that the viruses were of NDV.

**Real Time RT-PCR**

All 20 samples were assigned ct values ranging from 11.5 to 14.75 for ND screening in the Taqman qRT-PCR assay. Then, using a virulent F assay with ct values range of 12.30 to 15.50, all of the samples were determined to be virulent ND.

**Amplification and Sequencing of Fusion protein gene**

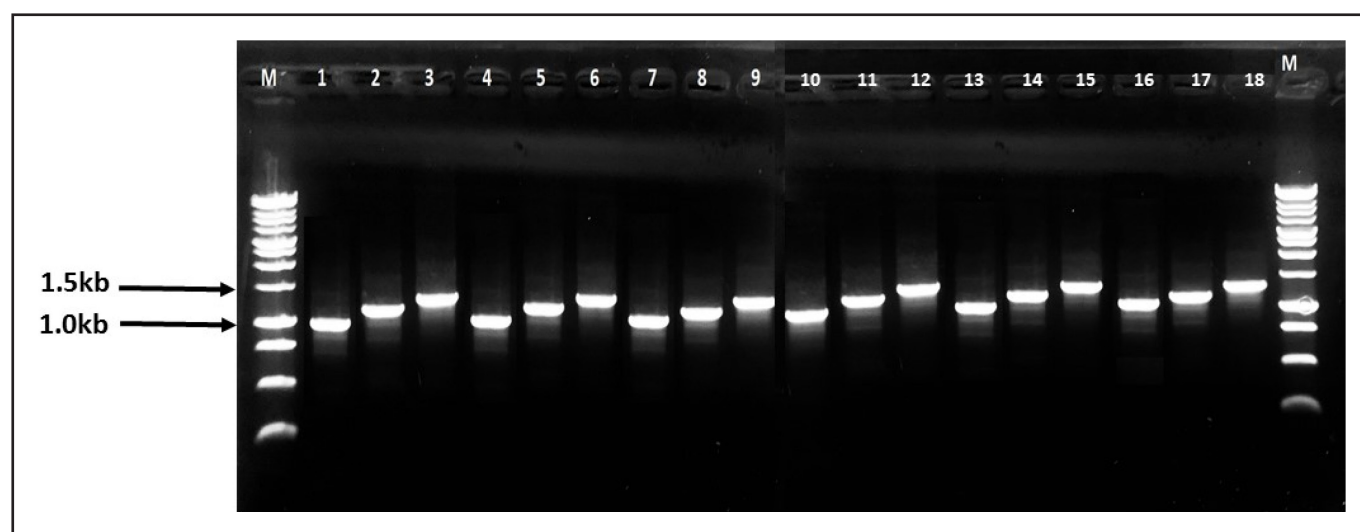
Six out 20 isolates were chosen for further molecular characterization of full length of F gene of the NDV. The isolates were chosen from the first positive case of different parts of Sabah; the Papar, Kota Kinabalu, Ranau, Penampang, Sandakan and Tawau. All six isolates were successfully amplified with complete F gene specific primers (Figure 2).

**Sequence and Phylogenetic Analysis**

The F gene’s 1662 bp nucleotide sequence was compared to other published sequences using data from the GenBank database. Sequence comparison among the Sabah isolates in this study were between 99.8 to 100%. Homology between these isolates and other published Malaysian NDV strains were between 90.00 to 91.5%. Isolates from this study shared 96.3 to 99.4% homology with strains from sub-genotype VII 1.1 (Table 3). Basic Local Alignment Search Tool (BLAST) results has shown that all isolates had 99.4% nucleotide homology with strain Ck/IR/MAM72/2018 from Iran isolated in 2018.

**Table 1.** Primer sets used in the amplification of complete F gene (Gould *et al.*, 2003)

Name	Sequences 5’ – 3’	Size of amplicon (bp)
NDV-4358F	GCA CAC CCT TGC CAA ATA CAA TCC	950
NDV-5307R	AAC TTA GTC AAT AAG TAA TCC AT	
NDV-4701F	CCT CAT CCC AGA CAG G	1149
NDV-5849R	AAA GTT ATC CCG TCT AAG GAT AA	
NDV-5724F	AGA TGA CAA CAT GTA GAT G	1298
NDV-7021R	GAA TGT GAG TGA TCT CTG CA	



**Figure 2.** RT-PCR amplification of full length of 6 isolates in this study. Amplification products of full length F gene using 3 primer sets NDV-4358F/ NDV-5307, NDV-4701F/ NDV-5849R and NDV-5724F/ NDV-7021R respectively. Lane 1 to 3: VRI-101-2021; Lane 4 to 6: VRI-751-2021; Lane 7 to 9: VRI-752-2021; Lane 10 to 12: VRI-753-2021; Lane 13 to 15: VRI-758-2021; Lane 16 to 18: VRI-763-2021. M: 1kb DNA ladder marker.

**Table 2.** List of isolates used in this study including the FO cleavage motif and BLAST results

No.	DI No	Date Received	Area	Breed	Vaccination status	BLAST Analysis			FO Cleavage site motif	Virulence	Accession Number
						Highest Nucleotide Homology	% Nucleotide Identity				
1.	VRI-101-2021	13/1/2021	Papar	Ross 308	Vaccinated	Ck/IR/MAM72/2018	99.4	RRQKRF	Virulent	OK338510	
2.	VRI-751-2021	8/2/2021	Kota Kinabalu	Ross 308	Vaccinated	Ck/IR/MAM72/2018	99.4	RRQKRF	Virulent	OK338511	
3.	VRI-752-2021	8/2/2021	Penampang	Ross 308	Vaccinated	Ck/IR/MAM72/2018	99.4	RRQKRF	Virulent	OK338512	
4.	VRI-753-2022	8/2/2021	Ranau	Ayam kampung	Not vaccinated	Ck/IR/MAM72/2018	99.4	RRQKRF	Virulent	OK338513	
5.	VRI-758-2027	8/2/2021	Sandakan	Broiler	Vaccinated	Ck/IR/MAM72/2018	99.4	RRQKRF	Virulent	OK338514	
6.	VRI-763-2032	8/2/2021	Tawau	Fighting cock	Not vaccinated	Ck/IR/MAM72/2018	99.4	RRQKRF	Virulent	OK338515	

Legend: R: Arginine; Q: Glutamine; K: Lysine; F Phenylalanine.

**Table 3.** Evolutionary distances of nucleotide sequences estimated between the mean distances (lower diagonal) and sequence homology comparison (upper diagonal) of six isolates with subgenotypes VII 1.1 and VII.2 of the fusion (F) gene

Isolates	1	2	3	4	5	6	7	8	9	10	11	12	13	14
1. VRI-101-2021		99.9	100	100	100	99.9	91.4	91.2	91.5	90.3	90.0	96.3	96.3	99.4
2. VRI-751-2021	0.001		99.9	99.9	99.9	100	91.3	91.1	91.5	90.3	90.0	96.2	96.3	99.3
3. VRI-752-2021	0.000	0.001		100	100	99.9	91.4	91.2	91.5	90.3	90.0	96.3	96.3	99.4
4. VRI-753-2021	0.000	0.001	0.000		100	99.9	91.4	91.2	91.5	90.3	90.0	96.3	96.3	99.4
5. VRI-758-2021	0.000	0.001	0.000	0.000		99.9	91.4	91.2	91.5	90.3	90.0	96.3	96.3	99.4
6. VRI-763-2021	0.001	0.000	0.001	0.001	0.001		91.3	91.1	91.5	90.3	90.0	96.2	96.3	99.3
7. KF026013 UPM-IBS/002/2011 Genotype VII.2(VIIh)	0.085	0.086	0.085	0.085	0.085	0.086		99.6	99.8	91.5	91.8	93.2	92.7	91.9
8. KR074405 IBS005/11 Genotype VII.2 (VIIh)	0.088	0.088	0.088	0.088	0.088	0.088	0.004		99.6	91.5	91.8	92.9	92.5	91.6
9. ON210972 chicken/Selangor/8857/2019 Genotype VII.2 (VIIh)	0.084	0.085	0.084	0.084	0.084	0.085	0.001	0.004		91.6	91.9	93.3	92.9	92.0
10. ON210971 chicken/Sabah/1502/2019 Genotype VII.2 (VIIi)	0.096	0.097	0.096	0.096	0.096	0.097	0.084	0.084	0.083		97.1	92.1	91.7	90.4
11. ON210972 chicken/Sabah/9457/2019 Genotype VII.2 (VIIi)	0.099	0.100	0.099	0.099	0.099	0.100	0.082	0.082	0.081	0.028		92.2	91.8	90.3
12. FJ872531 Muscovy_duck/China(Fujian)/FP1/02 Genotype VII 1.1 (VIId)	0.037	0.037	0.037	0.037	0.037	0.037	0.068	0.070	0.067	0.078	0.077		98.6	96.6
13. KU710279 chicken/Ukraine/Lugansk/2003 Genotype VII 1.1 (VIId)	0.036	0.037	0.036	0.036	0.036	0.037	0.072	0.075	0.071	0.082	0.081	0.014		96.5
14. MH481362 Ck/IR/MAM72/2018 Genotype VII 1.1 (VIIi)	0.005	0.006	0.005	0.005	0.005	0.006	0.081	0.083	0.079	0.095	0.097	0.034	0.034	

The F protein cleavage site for all the isolates in this study revealed the presence of multiple basic amino acid residues at positions 112, 113, 115 and 116 and phenylalanine (F) at position 117 (<sup>112</sup>RRQKR<sup>117</sup>) representing the characteristic for virulent ND (Figure 3).

Results of the phylogenetic analysis (Figure 4) showed that all isolates were clustered as Class II, genotype VII and were grouped under sub-genotype VIII which are phylogenetically close to NDV isolates from Iran. However, under the updated classification, all these isolates fall under sub-genotype VII 1.1. Evolutionary distance between the isolates revealed low distance score of 0 to 0.1%. In addition, isolates in this study and other published Malaysian NDV (sub-genotype VII.2) isolates had evolutionary distance ranging from 8.4 to 10%. Meanwhile, compared to strains from sub-genotype VII 1.1, isolates in this study had lower score of evolutionary distance with values between 0.6 to 3.7% (Table 3).

### DISCUSSION

Here we report the isolation and molecular characterization of NDV isolated from outbreaks in six different areas in Sabah during early 2021. Based on our findings, all six isolates are very much identical as they shared high percentage of similarities and likely to share a common origin.

Phylogenetic analysis clearly indicated that isolates in this study were clustered under genotype VII. In the early 1990s, genotype VII appeared in Europe and East Asia (Lomniczi *et al.*, 1998). Since then, genotype VII has become the most prevalent genotype that has caused NDV outbreaks all around the world including Malaysia (Miller *et al.*, 2015; Shohaimi *et al.*, 2015; Aljumaili *et al.*, 2017; Syamsiah *et al.*, 2021). Over the last two decades, genotype VII has been consistently detected in commercial and backyard poultry in Malaysia, and currently it is the predominant pathogen causing majority of ND outbreaks in this country in recent years. (Berhanu *et al.*, 2010; Tan *et al.*, 2010; Shohaimi *et al.*, 2015; Aljumaili *et al.*, 2017).

Genotype VII comprises highly diverse viruses. Therefore, based on the complete F sequence, Diel *et al.* (2012) had further divided the viruses into sub-genotypes; VIIa to VIII. A study by Shohaimi *et al.* (2015) reported that VIIb and VIIe were detected in 1999 while VIId was identified to be circulated in Malaysia between the year 2000 to 2009. This is before VIIh and VIIi become the predominant sub-genotypes and has been enzootic not only in Malaysia but also in Southeast Asia countries (Miller *et al.*, 2015; Liu *et al.*, 2019).

Although ND is endemic in Malaysia, previous reports revealed that NDV isolated in Sabah has been seen only sporadically and did not cause major outbreaks (Berhanu *et al.*, 2010; Shohaimi *et al.*, 2015; Syamsiah *et al.*, 2021). To our knowledge, this is the first time ND outbreaks were reported in many areas in Sabah including commercial farms. Based on the scale of the outbreaks, it was speculated that it could be due to a new genotype or sub-genotype. As described by Dimitrov *et al.* (2019), evolutionary distance below 5% are considered same sub-genotype. Our study showed that recent Sabah isolates and other previous published Malaysian isolates from genotype VII.2 (VIIh and VIIi) had evolutionary distance of more than 5% (8.4 to 10%) indicating that isolates in this study are in different sub-genotype. Furthermore, our BLAST results showed that the recent Sabah isolates had high genetic similarities with isolate from Iran isolated in 2018. Consistent with the finding, the evolutionary distance between isolates in this study and isolates from genotype VII 1.1 (VIId and VIII) were below than 5% suggesting that they are of the same sub-genotype.

Based on the phylogenetic analysis, all isolates from this study were segregated into sub-genotype VII 1.1 and clustered together with isolates from Iran (previously known as sub-genotype VIII). It can thus be suggested that there is an emerging of a new sub-genotype into the poultry population in Sabah and this sub-genotype has never been reported before in Malaysia. Interestingly, sub-genotype VIII seems to be very unique to Iran. It has been found in commercial broiler farms as well as backyard poultry (Molouki *et al.*, 2019). This sub-genotype was previously thought of as sub-genotype VIId due to different genotyping systems used in various studies (Sabouri *et al.*, 2018; Molouki *et al.*, 2021a). Sabouri *et al.* (2018) firstly reported that this genotype was identified in backyard poultry in Iran during 2011–2013. It was later detected in many commercial broiler farms from 2017 to 2020 (Molouki *et al.*, 2019; Allahyari *et al.*, 2020; Molouki *et al.*, 2021a). This is similar to our findings where most of the cases were isolated from commercial broilers. Areas affected by ND outbreaks are where most of the commercial poultry farms in Sabah are located (Anon, 2019). In Iran, this sub-genotype was also detected in other avian species such as pigeons, magpie, pheasant, turkey, peacock and ostrich (Khosravi *et al.*, 2021; Molouki *et al.*, 2021a, 2021b). Since then, this sub-genotype is considered as the predominant sub-genotype in Iran (Khosravi *et al.*, 2021; Molouki *et al.*, 2021b).

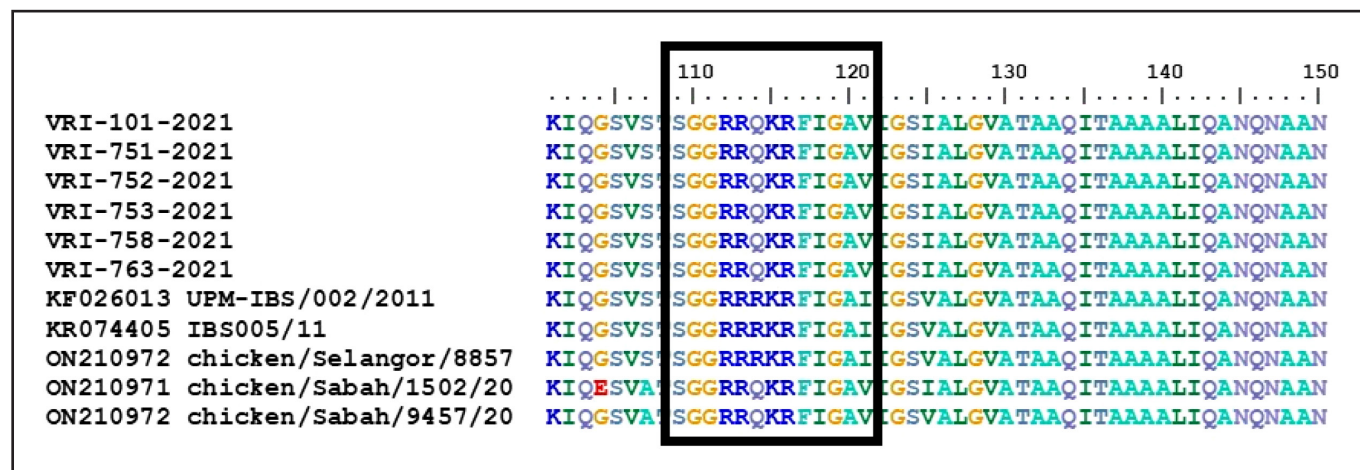
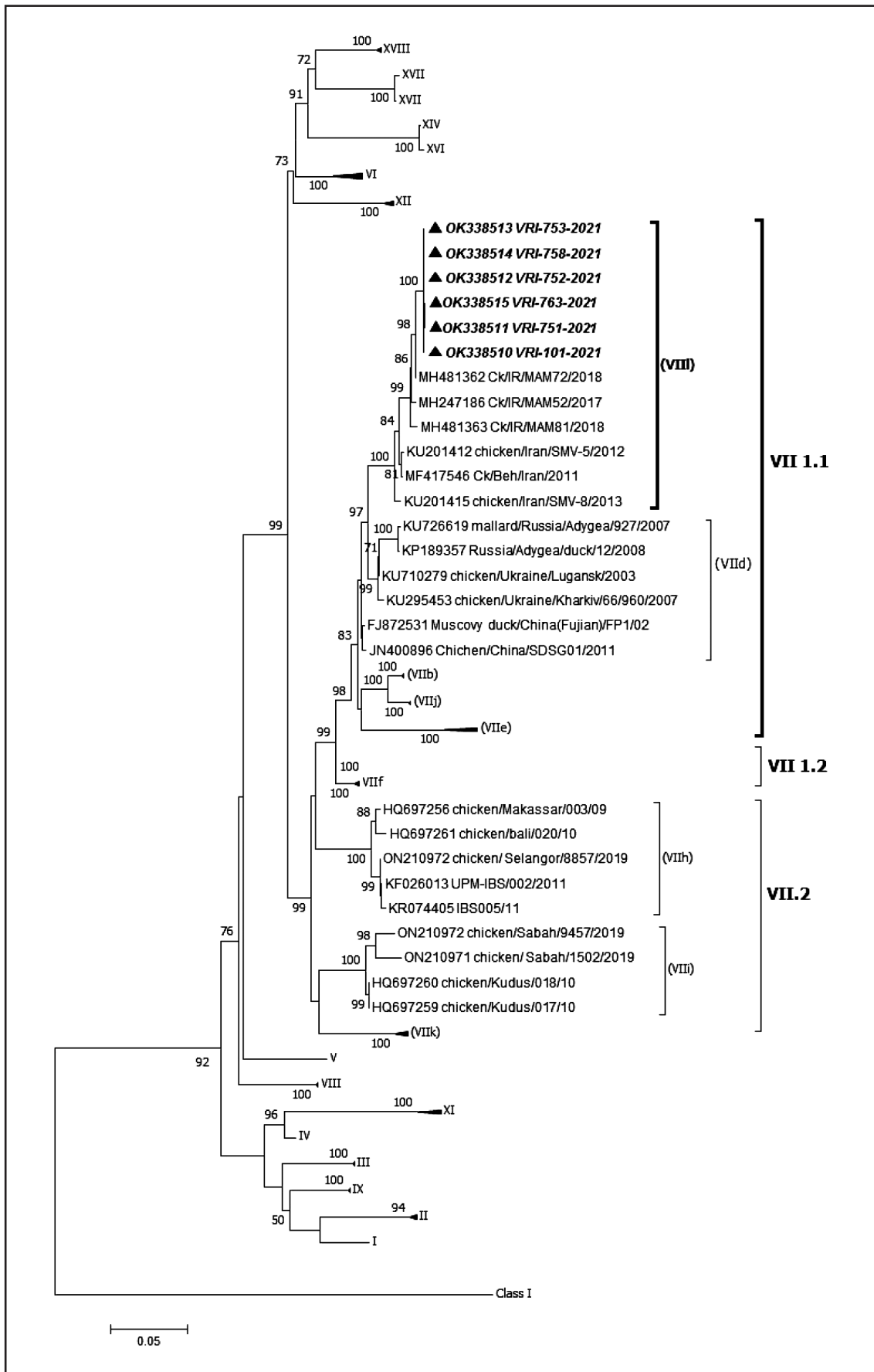


Figure 3. Alignment of the deduced amino acid sequences of the F gene of NDV isolates from Sabah compared with other Malaysian isolates from Genbank. The F protein cleavage site amino acid (residue 112 to 117) sequences of NDV isolates were labelled by a black rectangle.



**Figure 4.** Phylogenetic tree constructed on the basis of complete fusion gene sequences of NDV isolates obtained from this study and other NDV isolates representing different genotypes and sub-genotypes of Class I and Class II. Genotypes are according to Dimitrov *et al.* (2019). Genotypes according to Diel *et al.* (2012) are shown in parenthesis. Tree was constructed using MEGA version 7.0.26 by maximum likelihood (ML) statistical method with Kimura 2-parameter model and setting bootstrap 1000 replicates. The black triangle (▲) represents the isolates in this study.

Based on updated classification by Dimitrov *et al.* (2019), genotype VII is classified into three sub-genotypes, VII.1.1, VII.1.2, and VII.2. Therefore, the six isolates in this study are reclassified under sub-genotype VII.1.1. The viruses under sub-genotype VII.1.1 caused the fourth NDV panzootic. Meanwhile, sub-genotype VII.2 is viruses that were responsible for the fifth NDV panzootic (Dimitrov *et al.*, 2019).

Based on this new definition, since 2010, NDV that cause outbreaks in Malaysia is due to sub-genotype VII.2. Meanwhile, sub-genotype VII.1.1 has not been detected in Malaysia after 2009. It is worth to note that the strain that was circulating in Malaysia back then (2000 to 2009), was closely related to the China strain (sub-genotype VIId) (Shohaimi *et al.*, 2015). Previous studies reported, the last sub-genotype VIId isolated in Sabah was in 2005 (Berhanu *et al.*, 2010; Shohaimi *et al.*, 2015). This was before sub-genotype VII.2 emerged as the outbreak's causal strain in Malaysia (Shohaimi *et al.*, 2015). Based on a study in 2019, only sub-genotype VII.2 (VIIi) was isolated in Sabah (Syamsiah *et al.*, 2021). In addition to that, similar sub-genotype were also reported circulating in surrounding countries such as Indonesia and Philippines (Huong Tran *et al.*, 2021; Saputri *et al.*, 2021). Therefore, the source of introduction of this sub-genotype VII.1.1 remained unknown.

Several possible reasons may cause the introduction of this sub-genotype. Firstly, is the spillover of sub-genotype VII.1.1 virus from wild birds into poultry (Khosravi *et al.*, 2021). In Iran, other than commercial and backyard chickens, sub-genotype VII.1.1 was also isolated in other avian species (Khosravi *et al.*, 2021; Molouki *et al.*, 2021a, 2021b). Bidirectional spillovers occasionally disseminate virulent strains among wild bird species and chicken populations. Therefore, infected wild bird reservoirs may have crucial roles in introducing NDV to susceptible hosts. Unvaccinated birds, particularly backyard chickens and non-poultry avian species, have a high chance of coming into contact with wild birds due to a lack of biosecurity measures (Brown *et al.*, 2017). As a result, they could act as an amplification host if they are exposed to virulent strains of NDV. This is in agreement with our findings where two out of six virulent NDVs were isolated from village chicken and fighting cock. Furthermore, backyard birds with other non-poultry avian species kept for other reasons are always present surrounding commercial poultry farms (Hussain *et al.*, 2020). Hence, it increases the probability of NDV transmission to commercial poultry farms (Hussain *et al.*, 2020). Trade and/or illegal movement of poultry products and birds cannot be ruled out as a method of introduction of the NDV strains in this study (Dimitrov *et al.*, 2016). Although plausible, we had inadequate evidence to support any of these assumptions.

The virus spreads rapidly by the movement of people as well as contaminated equipment, food and water (Schirrmacher, 2020). Human activities mainly through the movement of live birds, fomites, personnel, and poultry products from infected premises to susceptible birds have been shown to result in spillover events (Brown *et al.*, 2021). Therefore, the human role might have contributed to the spread of sub-genotype VII.1.1 internally within the farms (Molouki *et al.*, 2021a). This is because, though isolates from this study were recovered from different geographical areas in Sabah, all isolates showed high genetic similarity.

From the results of this study, NDV was also isolated in a diseased chicken belonging to flocks that had received ND vaccination. Even though this is a new emerging sub-genotype in Sabah, and Malaysia generally, according to Miller *et al.* (2015), all NDV are of a single serotype, and any ND vaccine strain should protect against NDV-related mortality and morbidity regardless of the genotype differences. Nevertheless, vaccines must be used in conjunction with appropriate sound biosecurity, better-programmed strategies and good sanitary measures for the control program to be successful (Brown *et al.*, 2021). However, the exact reason for the occurrence of ND outbreaks in vaccinated flocks should be further

investigated. Furthermore, ongoing surveillance of the disease in various bird species, as well as joint efforts between farmers and local authorities, would help in the control of future ND outbreaks.

## CONCLUSION

In conclusion, this study has identified NDV sub-genotype VII.1.1 that causes the recent ND outbreaks in Sabah. This strain is genetically close to Iran isolates (previously known as sub-genotype VIII). This is a new introduction of the NDV sub-genotype VII.1.1 in Malaysia. The study's findings recommend for optimal transboundary monitoring and continuous surveillance to ensure proper disease control and prevention. Further molecular epidemiological analyses of NDVs are needed in order to understand the circulatory patterns of virulent strains of NDV in the country.

## ACKNOWLEDGEMENTS

We would like to thank the Director General of Veterinary Services Malaysia and Director of Veterinary Research Division for granting us permission to publish the paper. A great appreciation to the Sabah DVS and Veterinary Diagnostic Laboratory Kota Kinabalu, Sabah for providing the samples. Financial support for this research was provided by the Veterinary Research Institute, Ipoh Malaysia.

## Conflict of interest

The author declares that they have no conflict of interests.

## REFERENCES

- Alexander, D.J., Aldous, E.W. & Fuller, C.M. (2012). The long view: a selective review of 40 years of Newcastle disease research. *Avian Pathology* **41**: 329-335. <https://doi.org/10.1080/03079457.2012.697991>
- Aljumaili, O.A., Yeap, S.K., Omar, A.R. & Ideris, A. (2017). Isolation and characterization of genotype VII Newcastle disease virus from NDV vaccinated farms in Malaysia. *Pertanika Journal of Tropical Agricultural Science* **40**: 677-690.
- Allahyari, E., Allymeh, M., Molouki, A., Fallah Mehrabadi, M.H. & Talebi, A. (2020). Molecular characterisation and phylogenetic study of the fusion gene of Newcastle disease viruses isolated from broiler farms of Iran in 2018–2019. *Bulgarian Journal of Veterinary Medicine* **25**: 21-32. <https://doi.org/10.15547/bjvm.2020-0041>
- Amarasinghe, G.K., Archiga Ceballos, N.G., Banyard, A.C., Basler, C.F., Bavari, S., Bennett, A.J., Blasdel, K.R., Briese, T., Bukreyev, A., Cai, Y. *et al.* (2018). Taxonomy of the order Mononegavirales: update 2018. *Archives of Virology* **163**: 2283-2294. <https://doi.org/10.1007/s00705-018-3814-x>
- Anonymous (2019). Industri Ternakan. Jabatan Perkhidmatan Sabah. <https://vet.sabah.gov.my/content/industri-ternakan>. Accessed 21 October 2022
- Berhanu, A., Ideris, A., Omar, A.R. & Bejo, M. (2010). Molecular characterization of partial fusion gene and C-terminus extension length of haemagglutinin-neuraminidase gene of recently isolated Newcastle disease virus isolates in Malaysia. *Virology Journal* **7**: 183-192.
- Brown, I.H., Cargill, P.W., Woodland, R.M. & van den Berg, T. (2021). Newcastle Disease Virus. In: *Veterinary Vaccines: Principles and Applications*, S. Metwally, A., El Idrissi & Viljoen, G. (editors) First edition. New York: John Wiley & Sons Ltd, pp. 335-353.
- Brown, V.R. & Bevins, S.N. (2017). A review of virulent Newcastle disease viruses in the United States and the role of wild birds in viral persistence and spread. *Veterinary Research* **48**: 68. <https://doi.org/10.1186/s13567-017-0475-9>
- Diel, D.G., da Silva, L.H., Liu, H., Wang, Z., Miller, P.J. & Afonso, C.L. (2012). Genetic diversity of avian paramyxovirus type 1: proposal for a unified nomenclature and classification system of Newcastle disease virus genotypes. *Infection, Genetics and Evolution* **12**: 1770-1779. <https://doi.org/10.1016/j.meegid.2012.07.012>
- Dimitrov, K.M., Bolotin, V., Muzyka, D., Goraichuk, I.V., Solodiantkin, O., Gerilovych, A., Stegnyy, B., Goujgoulova, G.V., Silko, N.Y., Pantin-Jackwood, M.J. *et al.* (2016). Repeated isolation of virulent Newcastle disease viruses of sub-genotype VIId from backyard chickens in Bulgaria and Ukraine between 2002 and 2013. *Archives of Virology*, **161**: 3345-3353. <https://doi.org/10.1007/s00705-016-3033-2>

- Dimitrov, K.M., Abolnik, C., Afonso, C.L., Albina, E., Bahl, J., Berg, M. & Wong, F.Y.K. (2019). Updated unified phylogenetic classification system and revised nomenclature for Newcastle disease virus. *Infection, Genetics and Evolution* **74**: 103917. <https://doi.org/10.1016/j.meegid.2019.103917>
- Ganar, K., Das, M., Sinha, S. & Kumar, S. (2014). Newcastle disease virus: current status and our understanding. *Virus Research* **184**: 71-81. <https://doi.org/10.1016/j.virusres.2014.02.016>
- Ghalyanchilangeroudi, A., Hosseini, H., Jabbarifakhr, M., Fallah Mehrabadi, M.H., Najafi, H., Ghafouri, S.A., Mousavi, F.S., Ziafati, Z. & Modiri, A. (2018). Emergence of a virulent genotype VIII of Newcastle disease virus in Iran. *Avian Pathology* **47**: 509-519. <https://doi.org/10.1080/03079457.2018.1495313>
- Gould, A.R., Hansson, E., Selleck, K., Kattenbelt, J.A., Mackenzie, M. & Della-Porta, A. J. (2003). Newcastle disease virus fusion and haemagglutinin-neuraminidase gene motifs as markers for viral lineage. *Avian Pathology* **32**: 361-373. <https://doi.org/10.1080/0307945031000121112>
- Hall, T.A. (1999). BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. *Nucleic Acids Symposium Series* **41**: 95-98.
- Huong Tran, G.T., Mananggit, M.R., Bilbao Abao, L.N., Van Dong, H., Takeda, Y., Ogawa, H. & Imai, K. (2021). Molecular characterization of a newcastle disease virus isolate from a diseased chicken in the philippines in 2017. *Japanese Journal of Veterinary Research* **69**: 73-81. <https://doi.org/10.14943/jjvr.69.1.73>
- Hussain, A., Wajid, A., Ather, S., Alyas, K., Awais, M., Khan, M.R., Hussain, T. & Babar, M.E. (2020). Isolation and genetic characterization of virulent strains of avian paramyxovirus-1 from multiple avian species in Azad Jammu and Kashmir 2017-2018. *Brazilian Journal of Microbiology* **51**: 385-394. <https://doi.org/10.1007/s42770-019-00193-0>
- International Committee on Taxonomy of Viruses. (2019). Virus taxonomy. <https://talk.ictvonline.org/taxonomy/> Accessed 21 Oct 2022.
- Khosravi, M., Seifi, S. & Tazeh, Z. (2021). Molecular characterization and phylogenetic analysis of VIII sub-genotype of avian orthoavulavirus 1 isolated from Eurasian magpie (*Pica pica*). *Iranian Journal of Veterinary Research* **22**: 155-160. <https://doi.org/10.22099/ijvr.2021.37584.5470>
- Liu, H., Wang, J., Ge, S., Lv, Y., Li, Y., Zheng, D. & Wang, Z. (2019). Molecular characterization of new emerging sub-genotype VIIIh Newcastle disease viruses in China. *Virus Genes* **55**: 314-321. <https://doi.org/10.1007/s11262-019-01651-5>
- Lomniczi, B., Wehmann, E., Herczeg, J., Ballagi-Pordány, A., Kaleta, E.F., Werner, O., Meulemans, G., Jorgensen, P.H., Manté, A.P., Gielkens, A.L. *et al.* (1998). Newcastle disease outbreaks in recent years in western Europe were caused by an old (VI) and a novel genotype (VII). *Archives of Virology* **143**: 9-64. <https://doi.org/10.1007/s007050050267>
- Mahamad, S.N.A., Tan, S.W., Youn, S.-Y., Lee, H.-J., Lee, J.-Y., Kwon, Y.-K., Ideris, A., & Omar, A.R. (2021). Isolation and characterization of Newcastle Disease Virus subgenotype VII.2/VIII from commercial chicken and swan in Malaysia. *Pertanika Journal of Tropical Agricultural* **44**: 879-898. <https://doi.org/10.47836/PJTAS.44.4.10>
- Miller, P.J., Haddas, R., Simanov, L., Lublin, A., Rehmani, S.F., Wajid, A., Bibi, T., Khan, T.A., Yaqub, T., Setyaningsih, S. *et al.* (2015). Identification of new sub- genotypes of virulent Newcastle disease virus with potential panzootic features. *Infection, Genetics and Evolution* **29**: 216-229. <https://doi.org/10.1016/j.meegid.2014.10.032>
- Molouki, A., Sotani, M., Fallah Mehrabadi, M.H., Shoushtari, A., Abtin, A., Mahmoudzadeh Akhijahani, M., Abdoshah, M., Pourbakhsh, S.A., Allahyari, E., Ghalyanchilangeroudi, A. *et al.* (2021a). Predominance of fourth panzootic Newcastle Disease Virus Sub-genotype VII.1.1 in Iran and its relation to the genotypes circulating in the region. *Current Microbiology* **78**: 3068-3078. <https://doi.org/10.1007/s00284-021-02572-z>
- Molouki, A., Soltani, M., Akhijahani, M.M., Merhabadi, M., Abtin, A., Shoushtari, A., Langeroudi, A.G., Lim, S., Allahyari, E., Abdoshah, M. *et al.* (2021b). Circulation of at least six distinct groups of pigeon-derived Newcastle Disease Virus in Iran between 1996 and 2019. *Current Microbiology* **78**: 2672-2681. <https://doi.org/10.1007/s00284-021-02505-w>
- Molouki, A., Mehrabadi, M., Bashashati, M., Akhijahani, M.M., Lim, S. & Hajloo, S.A. (2019). NDV sub-genotype VII(L) is currently circulating in commercial broiler farms of Iran, 2017-2018. *Tropical Animal Health And Production* **51**: 1247-1252. <https://doi.org/10.1007/s11250-019-01817-1>
- Nagy, A., Ali, A., Zain El-Abideen, M.A., Kilany, W. & Elsayed, M. (2020). Characterization and genetic analysis of recent and emergent virulent newcastle disease viruses in Egypt. *Transboundary and Emerging Diseases* **1**: 1-13. <https://doi.org/10.1111/tbed.13543>
- NCBI Resource Coordinators (2016). Database resources of the National Center for Biotechnology Information. *Nucleic Acids Research* **44**(D1): D7-D19. <https://doi.org/10.1093/nar/gkv1290>
- OIE (World Organisation for Animal Health). (2021). OIE Terrestrial Manual. Chapter 3. 3.14 Newcastle disease (infection with Newcastle disease virus). [https://www.oie.int/fileadmin/Home/eng/Health\\_standards/tahm/3.03.14\\_NEWCASTLE\\_DIS.pdf](https://www.oie.int/fileadmin/Home/eng/Health_standards/tahm/3.03.14_NEWCASTLE_DIS.pdf). Accessed 21 October 2022.
- Peeters, B. & Koch, G. (2021). Newcastle Disease Virus (*Paramyxoviridae*). *Encyclopedia of Virology* (Fourth Edition) **2**: 648-653. <https://doi.org/10.1016/b978-0-12-809633-8.20933-3>
- Sabouri, F., Vasfi Marandi, M. & Bashashati, M. (2018). Characterization of a novel VIII sub-genotype of Newcastle disease virus circulating in Iran. *Avian Pathology* **47**: 90-99. <https://doi.org/10.1080/03079457.2017.1376735>
- Saputri, M.E., Poetri, O.N. & Soejoedono, R.D. (2021). Phylogenetic studies of Newcastle disease virus isolated from poultry flocks in South Sulawesi Province, Indonesia, in 2019. *Journal of Advanced Veterinary and Animal Research* **8**: 129-137. <https://doi.org/10.5455/javar.2021.h495>
- Shohaimi, S.A., Raus, R.A., Ong, G.H., Asmayatim, B.M., Nayan N. & Yusuf, A.M. (2015). Sequence and phylogenetic analysis of Newcastle disease virus genotype VII isolated in Malaysia during 1999-2012. *Jurnal Teknologi* **77**: 159-164.
- Syamsiah, A.S., Leow, B.L., Faizul Fikri, M.Y., Muhammad Redzwan, S., Ong, G.H. & Faizah Hanim, M.S. (2021). Molecular phylogenetics of Newcastle Disease Virus isolated from chickens in 2019. *Malaysian Journal Veterinary Research* **12**: 51-58
- Tamura, K., Glen Stecher, Daniel Peterson, Alan Filipksi & Sudhir Kumar. (2013) MEGA6: Molecular Evolutionary Genetics Analysis Version 6.0. *Molecular Biology and Evolution* **30**: 2725-2729. <https://doi.org/10.1093/molbev/mst197>
- Tan, S.W., Ideris, A., Omar, A.R., Yusoff, K. & Hair-Bejo, M. (2010). Sequence and phylogenetic analysis of Newcastle Disease Virus genotypes isolated in Malaysia between 2004 and 2005. *Archives of Virology*. **155**: 63-70. <https://doi.org/10.1007/s00705-009-0540-4>
- Twabela, A.T., Nguyen, L.T., Masumu, J., Mpoyo, P., Mpiana, S., Sumbu, J., Okamatsu, M., Matsuno, K., Isoda, N., Zecchin, B. *et al.* (2021). A new variant among Newcastle Disease viruses isolated in the Democratic Republic of the Congo in 2018 and 2019. *Viruses* **13**: 151. <https://doi.org/10.3390/v13020151>
- Wang, Y., Yu, W., Huo, N., Wang, W., Guo, Y., Wei, Q., Wang, X., Zhang, S., Yang, Z. & Xiao, S. (2017). Comprehensive analysis of amino acid sequence diversity at the F protein cleavage site of Newcastle disease virus in fusogenic activity. *PLoS ONE* **12**: e0183923. <https://doi.org/10.1371/journal.pone.0183923>
- Wise, M.G., Suarez, D.L., Seal, B.S., Pedersen, J.C., Senne, D.A., King, D.J., Kapczynski, D.R. & Spackman, E. (2004). Development of a real-time reverse-transcription PCR for detection of newcastle disease virus RNA in clinical samples. *Journal of Clinical Microbiology* **42**: 329-338. <https://doi.org/10.1128/JCM.42.1.329-338.2004>