JOURNAL OF ADVANCED VETERINARY AND ANIMAL RESEARCH
ISSN 2311-7710 (Electronic)
http://doi.org/10.5455/javar.2021.h495
A periodical of the Network for the Veterinarians of Bangladesh (BDvetNET)

March 2021
VOL 8, NO. 1, PAGES 129–137

ORIGINAL ARTICLE

Phylogenetic studies of Newcastle disease virus isolated from poultry flocks in South Sulawesi Province, Indonesia, in 2019

Meliana Eka Saputri[1], Okti Nadia Poetri[2], Retno Damajanti Soejoedono[2]
[1]Study Program Medical Microbiology, Faculty of Veterinary Medicine, IPB University, Bogor, Indonesia
[2]Department of Animal Diseases and Veterinary Public Health, Faculty of Veterinary Medicine, IPB University, Bogor, Indonesia

ABSTRACT

Objective: Indonesia is one of the Newcastle disease (ND) endemic countries in the world. An outbreak of the ND virus (NDV) was first reported in Indonesia in 1926. This study aimed to detect, isolate, and classify the NDV by molecular approaches from poultry farms in South Sulawesi Province of Indonesia in 2019.

Materials and Methods: As many as 36 pooling samples from the cloacal swab, trachea swab, proventriculus, and spleen tissues obtained from ND-suspected chickens were isolated in 11-day-old embryonated chicken eggs type-specific antibody-negative. The viruses were confirmed by reverse transcription-polymerase chain reaction (RT-PCR), followed by sequencing.

Results: The results showed that 18 out of 36 pooling samples were NDV-positive based on the isolation result and RT-PCR test. The sequencing results showed that 10 NDV isolates had a motif ^{112}R-R-Q-K-R-F^{117} in the fusion protein cleavage site region, which suggested that the NDV isolates were of virulent pathotype. The phylogenetic studies based on the *F* gene's partial nucleotide sequence classified the study isolates into NDV virus genotype/subgenotype VII.2.

Conclusion: These findings are expected to help provide the latest characteristic information of NDV in South Sulawesi Province to determine the seed vaccine for control strategies of ND.

ARTICLE HISTORY

Received August 22, 2020
Revised December 02, 2020
Accepted December 05, 2020
Published March 07, 2021

KEYWORDS

F gene; Newcastle disease virus; Phylogenetic analysis; Sequencing

Introduction

Newcastle disease (ND) is one of the fatal viral infections in poultry and it has become the primary attention in poultry farm business because it causes massive loss [1,2]. It is caused by an ND virus (NDV) or *Avian avulavirus 1* (commonly known as *Avian paramyxovirus* serogroup *A. paramyxovirus* type 1 species), *Avulavirus* genus of the family Paramyxoviridae [3–6]. The NDV is an enveloped pleomorphic ribonucleic acid (RNA) virus; the genome is unsegmented with a single-stranded and negative polarity [4,7].

The NDV genome has six open reading frames that encode nucleocapsid protein, phosphoprotein, matrix protein, fusion protein (F), hemagglutinin-neuraminidase (HN), and large RNA-directed RNA polymerase [7,8]. Genetically, NDV has been divided into two major clades, Class I and Class II. Class I NDV consists of single genotypes and three sub-genotypes [3] and are mostly avirulent viruses for chickens. Class I NDVs are generally found in wild birds, and their genetic diversity is lower than Class II [3]. Class II NDVs are divided into at least 20 genotypes (I–XXI), and many sub-genotypes, more diverse, and contain a range of avirulent to virulent NDV [3,5].

Clinical symptoms presented in chickens infected with NDV are divided into five NDV pathotypes, i.e., viscerotropic velogenic as extremely pathogenic with high mortality and intestinal hemorrhagic lesion characteristic; neurotropic velogenic with high mortality, respiration, and nerve intrusion; mesogenic with low mortality, respiration, and nerve intrusion; lentogenic with light clinical symptoms in the respiration lumen; and asymptomatic enteric with a subclinical enteritis infection [9].

In Indonesia, ND outbreaks have occurred since late 1926 [10]. NDV isolates from Indonesia have been typed as genotypes 1, II, VI, and VII based on the *fusion* (*F*) gene phylogeny [9-12]. Isolates belonging to sub-genotype VIIh

Correspondence Retno Damajanti Soejoedono ✉ retnodmail@yahoo.com Department of Animal Diseases and Veterinary Public Health, Faculty of Veterinary Medicine, IPB University, Bogor, Indonesia.

How to cite: Saputri ME, Poetri ON, Soejoedono RD. Phylogenetic studies of Newcastle disease virus isolated from poultry flocks in South Sulawesi Province, Indonesia, in 2019. J Adv Vet Anim Res 2021; 8(1):129–137.

and VIIi, now namely sub-genotype VII.2, have also been recently reported [3,11–13]. The NDV, which is an RNA virus, can mutate rapidly, giving rise to new sub-genotypes. This study aimed to characterize the recent NDV field isolates using the molecular approach and determine the genotype of NDV in South Sulawesi Province, Indonesia.

Materials and Methods

Ethical approval

The research was conducted following the regulations in Animal Health of Indonesian Law on Livestock and Animal Health (UU/18/2009, article 80).

Duration and samples

The research was carried out from July 2019 to March 2020. A total of 36 samples were isolated from troubled flocks displaying ND-like clinical signs and proventriculitis. Samples were obtained from commercial poultry flocks in the South Sulawesi Province from Maros (n = 3), Barru (n = 15), Pangkajene Kepulauan (n = 4), Sidrap (n = 9), Soppeng (n = 2), and Pinrang (n = 3). The samples were proventriculus and spleen tissues, cloacal swabs, and tracheal swabs. The field samples in this study are presented in Table 1.

Virus inoculation

Samples were inoculated in specific antibody-negative embryonated chicken eggs at 9–11 days of age in Medical Microbiology at the Faculty of Veterinary Medicine, IPB University, Bogor, Indonesia. The inoculums solution was made by mixing the tracheal swab, cloaca swab, and tissues samples with 1 ml PBS containing 10,000 IU/ml penicillin-streptomycin. As much as 100 µl inoculums solution was inoculated into the allantoic cavity of embryonated eggs and incubated at 37°C for 4 days. The allantoic fluid was collected after incubation and tested for the presence of the virus by a rapid hemagglutination (HA) test using 1% chicken red blood cells. The positive agglutinated allantoic fluid was retested again by reverse transcription-polymerase chain reaction (RT-PCR). The NDV isolates were kept at −80°C for further analysis.

RNA isolation

The isolation of RNA from field samples was carried out using the Viral Nucleic Acid Extraction Kit II (Geneaid®) according to the manufacturer's procedure. The extracted RNA was kept at −80°C for RT-PCR analysis.

Reverse transcription-polymerase chain reaction (RT-PCR)

The RT-PCR test used a My Taq™ OneStep RT-PCR kit (Bioline®) according to manufacturer's instruction. The primers used in this study are shown in Table 2 [14]. The RT-PCR test's first step contained a reverse transcriptase at 45°C for 20 min and initiation at 95°C for 1 min. Amplification was carried out with 40 cycles containing denaturation (94°C, 30 sec), annealing (56°C, 30 sec), and extension (72°C, 30 sec). The final extension was carried out at 72°C for 7 min. The amplicon was visualized with electrophoresis (100 V/30 min) on 1.5% agarose gel.

Sequencing and analysis

PCR product sequencing was carried out from the First BASE Laboratories Sdn Bhd (Malaysia). Nucleotide sequences were adjusted and analyzed using MEGA 7 software [15]. A 498 nucleotides fragment spanning the 16–513 bp region of the *F* gene was available and used in the molecular analysis. The ten NDV sequences' evolutionary relationship in this study was determined based on a neighbor-joining method with the value of 1,000 bootstrap replications. The percentage value of tree replication from taxa/isolate forming the same cluster on the bootstrap test with 1,000 replications was presented to be closed to the branch. The tree was drawn based on scales as the same branch length unit as the evolution distance used to determine the phylogenetic tree.

Results

Virus isolation

Pooled samples from 36 ND suspected chickens were inoculated into embryonated chicken eggs at 9–11 days old with three replication per pooling sample. A list of the samples is presented in Table 3. A total of 18 samples showed a positive growth of NDV in specific antibody-negative embryonated chicken eggs, and a rapid HA test confirmed the occurrence of NDV in the allantoic fluid.

RT-PCR

The RT-PCR assay detected the presence of NDV in the allantoic fluid with an amplicon of 535 bp. The findings revealed that 18 samples were positive for NDV out of 36 samples (Fig. 1). The positive samples were obtained from broiler chickens and laying hens in Maros, Barru, Pinrang, Pangkep, and Sidrap. Furthermore, one positive sample was collected from the duck in Sidrap, South Sulawesi Province.

Sequencing

Ten RT-PCR positive samples were selected for sequencing analysis. These are Broiler/Pinrang 1/2019 (Accession No. MW030438); Broiler/Barru 12/2019 (Accession No. MW 030439); Broiler/Barru 14/2019 (Accession No. MW0 30440); Broiler/Barru 9/2019 (Accession No. MW030441); Broiler/Maros 3/2019 (Accession No. MW030442); Broiler/

Table 1. Field samples for identification of ND viruses in South Sulawesi Province, Indonesia.

Farm	Species	Age	District	Population size	Clinical signs/gross lesion
Broiler/Maros 1/2019	Chicken	14 days	Maros	≤3,000	Respiratory disorder
Broiler/Maros 2/ 2019	Chicken	17 days	Maros	≤3,000	Respiratory disorder
Broiler/Maros 3/ 2019	Chicken	14 days	Maros	≤3,000	Respiratory disorder
Layer/Barru 1/2019	Chicken	≥50 weeks	Barru	≤800	Weakness, paralysed
Layer/Barru 2/2019	Chicken	≥21 weeks	Barru	≤700	Respiratory disorder
Layer/Barru 3/2019	Chicken	≥21 weeks	Barru	≤800	Respiratory disorder
Layer/Barru 4/2019	Chicken	28 weeks	Barru	≤1,000	Respiratory disorder, swollen head syndrome
Layer/ Barru 5/2019	Chicken	16 weeks	Barru	≤1,000	Respiratory disorder, swollen head syndrome
Layer/Barru 6/2019	Chicken	48 weeks	Barru	≤1,000	Torticolis, respiratory disorder, paralysed
Layer/Barru 7/2019	Chicken	21 weeks	Barru	≤1,000	Torticolis, respiratory disorder, paralysed
Layer/Barru 8/2019	Chicken	≥23 weeks	Barru	≤8,000	Weakness
Kampung/Soppeng 1/2019	Chicken	12 weeks	Soppeng	≤100	Respiratory disorder
Layer /Soppeng 2/2019	Chicken	20 weeks	Soppeng	1,000	Respiratory disorder
Broiler/Pinrang 1/2019	Chicken	25 days	Pinrang	3,000	Respiratory disorder, 200-300 chicken died in 1 day
Broiler/Pinrang 2/ 2019	Chicken	25 days	Pinrang	3,500	Respiratory disorder, mortality 90% in one week
Broiler/Sidrap 1/2019	Chicken	25 days	Sidrap	3,000	Respiratory disorder
Broiler/Sidrap 2/2019	Chicken	25 days	Sidrap	3,000	Respiratory disorder , mortality 70%.
Broiler/Sidrap 3/2019	Chicken	30 days	Sidrap	4,000	Respiratory disorder , swollen head syndrome.
Layer/Sidrap 4/2019	Chicken	21 weeks	Sidrap	5,000	Respiratory disorder , swollen head syndrome
Layer/Sidrap 5/2019	Chicken	20 weeks	Sidrap	5,000	Respiratory disorder, swollen head syndrome
Layer/Sidrap 6/2019	Chicken	12 weeks	Sidrap	≤5,000	Respiratory disorder, mortality 20 chicken died per days
Broiler/Sidrap 7/2019	Chicken	30 days	Sidrap	3,000	Stunted, weakness
Itik/Sidrap 8/2019	Duck	16 weeks	Sidrap	3,000	No clinical signs
Itik/Sidrap 9/2019	Duck	16 weeks	Sidrap	3,000	No clinical signs
Broiler/Barru 9/2019	Chicken	27 days	Barru	3,500	Respiratory disorder
Broiler/Barru 10/2019	Chicken	26 days	Barru	3,600	Respiratory disorder
Broiler/Pangkep 1/2019	Chicken	26 days	Pangkajene Kepulauan	3,000	Respiratory disorder
Broiler/Pangkep 2/2019	Chicken	26 days	Pangkajene Kepulauan	3,000	Respiratory disorder
Itik/Barru 11/2019	Duck	≥ 24 weeks	Barru	≤100	No clinical signs
Broiler/Barru 12/2019	Chicken	26 days	Barru	3,600	Respiratory disorder, mortality 50%
Broiler/Barru 13/2019	Chicken	26 days	Barru	3,600	Respiratory disorder, mortality 50%
Broiler/Barru 14/2019	Chicken	26 days	Barru	3,600	Respiratory disorder, mortality 50%
Broiler/Barru 15/2019	Chicken	26 days	Barru	3,600	Respiratory disorder, mortality 50%
Broiler/Pinrang 3/ 2019	Chicken	25 days	Pinrang	3,000	Respiratory disorder, mortality 90%
Broiler/Pangkep 3/2019	Chicken	26 days	Pangkajene Kepulauan	3,000	Respiratory disorder
Broiler/Pangkep 4/2019	Chicken	26 days	Pangkajene Kepulauan	3,000	Respiratory disorder

Table 2. Primers of *F* gene detection.

Primer	Target gene	Sequences	Position nucleotide	Base pair
Fus-535F	*Fusion* ND	5'-ATGGGCTCCAGACCTTCTACCA-3'	47–67	535
Fus-535R		5'-CTGCCACTGCTAGTTGTGATAATC-3'	557–81	

Adapted from Sarah et al. [12] and Radwan et al. [14].

Table 3. Results of virus isolation.

No	Name of Sample isolate	Rapid HA test	Condition of embryo	Result of RT PCR
1	Broiler/Maros 1/2019	+ (3/3)	Haemorrhage (3/3)	positive
2	Broiler/Maros 2/ 2019	+ (3/3)	Haemorrhage (3/3)	positive
3	Broiler/Maros 3/ 2019	+ (3/3)	Haemorrhage (3/3)	positive
4	Layer/Barru 1/2019	– (0/3)	No Lesion (0/3)	negative
5	Layer/Barru 2/2019	– (0/3)	No Lesion (0/3)	negative
6	Layer/Barru 3/2019	– (0/3)	No Lesion (0/3)	negative
7	Layer/Barru 4/2019	– (0/3)	No Lesion (0/3)	negative
8	Layer/ Barru 5/2019	– (0/3)	No Lesion (0/3)	negative
9	Layer/Barru 6/2019	– (0/3)	No Lesion (0/3)	negative
10	Layer/Barru 7/2019	– (0/3)	No Lesion (0/3)	negative
11	Layer/Barru 8/2019	– (0/3)	No Lesion (0/3)	negative
12	Kampung/Soppeng 1/2019	– (0/3)	No Lesion (0/3)	negative
13	Layer /Soppeng 2/2019	– (0/3)	No Lesion (0/3)	negative
14	Broiler/Pinrang 1/2019	+ (3/3)	Haemorrhage (3/3)	positive
15	Broiler/Pinrang 2/ 2019	+ (3/3)	Haemorrhage (3/3)	positive
16	Broiler/Sidrap 1/2019	+ (1/3)	Haemorrhage (1/3)	positive
17	Broiler/Sidrap 2/2019	– (0/3)	No Lesion (0/3)	negative
18	Broiler/Sidrap 3/2019	– (0/3)	No Lesion (0/3)	negative
19	Layer/Sidrap 4/2019	– (0/3)	No Lesion (0/3)	negative
20	Layer/Sidrap 5/2019	– (0/3)	No Lesion (0/3)	negative
21	Layer/Sidrap 6/2019	+ (3/3)	Haemorrhage (3/3)	posistive
22	Broiler/Sidrap 7/2019	– (0/3)	No Lesion (0/3)	negative
23	Itik/Sidrap 8/2019	– (0/3)	No Lesion (0/3)	negative
24	Itik/Sidrap 9/2019	+ (2/3)	Stunted (2/3)	positive
25	Broiler/Barru 9/2019	+ (3/3)	No Lesion (3/3)	positive
26	Broiler/Barru 10/2019	– (0/3)	No Lesion (0/3)	negative
27	Broiler/Pangkep 1/2019	+ (3/3)	Haemorrhage (3/3)	positive
28	Broiler/Pangkep 2/2019	+ (3/3)	Haemorrhage (3/3)	positive
29	Itik/Barru 11/2019	– (0/3)	No Lesion (0/3)	negative
30	Broiler/Barru 12/2019	+ (3/3)	Haemorrhage (3/3)	positive
31	Broiler/Barru 13/2019	+ (3/3)	Haemorrhage (3/3)	positive
32	Broiler/Barru 14/2019	+ (3/3)	Haemorrhage (3/3)	positive
33	Broiler/Barru 15/2019	+ (3/3)	Haemorrhage (3/3)	positive
34	Broiler/Pinrang 3/2019	+ (3/3)	Haemorrhage (3/3)	positive
35	Broiler/Pangkep 3/2019	+ (3/3)	Haemorrhage (3/3)	positive
36	Broiler/Pangkep 4/2019	+ (3/3)	Haemorrhage (3/3)	positive

Maros 2/2019 (Accession No. MW030443); Broiler/ Maros 1/2019 (Accession No. MW030444); Broiler/ Pangkep 2/2019 (Accession No. MW030445); Layer/ Sidrap 6/2019 (Accession No. MW030446); and Itik/ Sidrap 9/2019 (Accession No. MW030447).

The phylogenetic analysis showed that all the ten field isolates of the NDV were clustered into genotype VII.2 (Fig. 2). Based on the sequencing result of 10 NDV, the comparison of the nucleotide sequence in *F* gene between virulent NDV and the previous NDV isolates from Indonesia (GenBank database) indicated that the NDV field isolates from South Sulawesi Province in 2019 have a nucleotide sequence similarity between 87.82% and 97.96% with NDV under sub-genotype VII.2 (Table 4).

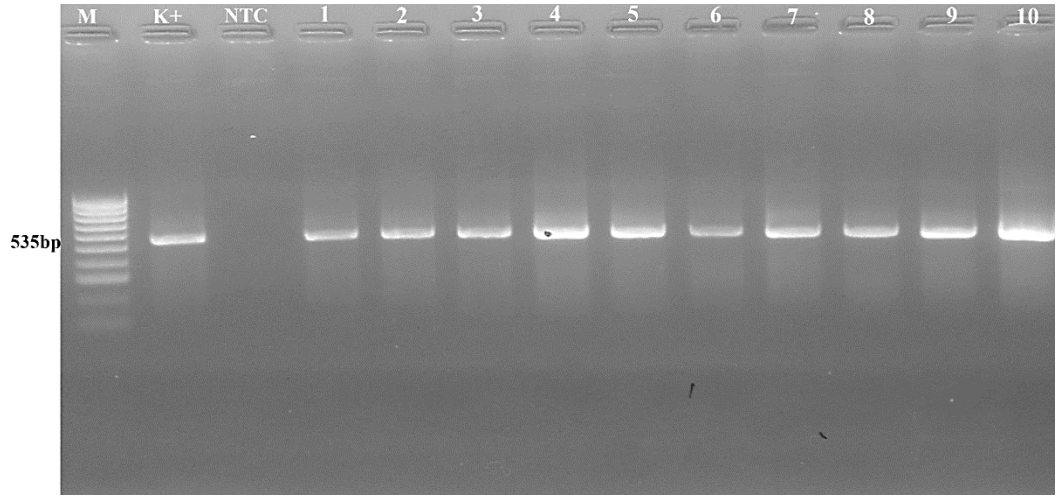

Figure 1. Amplification of 535-bp fragment of *F* gene of NDV by RT-PCR. **(**1) Broiler/Pinrang 1/2019; (2) Broiler/Barru 12/2019; (3) Broiler/Barru 14/2019; (4) Broiler/Barru 9/2019; (5) Broiler/Maros 3/2019 ; (6) Broiler/Maros 2/2019; (7) Broiler/Maros 1/2019; (8) Broiler/Pangkep 2/2019; (9) Layer/Sidrap 6/2019; and (10) Itik/Sidrap 9/2019; K+ Positive control; NTC Non template control. Bioline 100-bp was used as marker.

Analysis of the F cleavage site in this study showed that all isolates had the same motif, namely ^{112}R-R-Q-K-R-F^{117} (Fig. 3). Virulent NDV possesses multiple arginines (R) or lysine (K) residues in the cleavage site region of F protein at 112–7; therefore, the isolates could be velogenic pathotypes of NDV.

Discussion

Indonesia is one of the ND endemic countries. The first case of ND was reported in Java Province, Indonesia, in 1926 and spread to all the provinces of Indonesia. Poor biosecurity and inadequate control strategies are the leading causes of NDV transmission in commercial poultry and wild birds [15]. Putri et al. [11] noted that in recent years, there are two genotypes of NDV that are always present in Indonesia; these are genotype II and genotype VII. The NDV belonging to the sub-genotype VII.2 is known as the fundamental cause of infectious ND at poultry in Indonesia [10,11], and genotype II NDV, in particular, LaSota, is used as a seed in a live vaccine [12].

The ten samples in this study suggested a high virulent virus indicated by their amino acid sequences in the F cleavage site. The F cleavage site region of virulent NDV has at least three basic amino acid residues (polybasic cleavage site) of arginine (R) or lysine (K) at 112–6 position and phenylalanine (F) at 117 positions. The avirulent NDV has some parameters, including that these have less than three basic amino acids (monobasic cleavage site) at 112–6 positions and leucine (L) at 117 positions [16]. All isolates of NDV from this study were identified to have a similar amino acid motif on the cleavage site region of the F protein identical to other genotypes VII.2 viruses, i.e., ^{112}R-R-Q-K-R-F^{117}, which is included in one group of virulent NDV. The virulent pathotype of NDV isolates should be confirmed by a biological test such as mean death time on the chicken embryo, intracerebral pathogenicity index, and intravenous pathogenicity index [16,17].

A phylogenetic tree was used to observe the genotypic or sub-genotypic classification of NDV, and all field NDV isolates analyzed in this study were included in sub-genotype VII.2 (Fig. 3). Some nucleotide sequence data of NDV available in the GenBank can be used as a reference to compare the nucleotide sequences and carry out a phylogenetic analysis for predicting the genotype and determining the virus origin causing ND in a region.

The NDV isolates have been categorized into class I and class II. Class I isolates are commonly isolated from wild birds and domesticated poultry in Africa, Asia, Europe, and the USA. Most members of this class have low virulence abilities for chickens. On the contrary, class II isolates have a higher genetic diversity, ranging from avirulent and vaccine strains to highly virulent strains [3,18].

The NDV included in genotypes I–IV were discovered before 1960, while genotypes V, VI, and VII were known to infect in 1980 and cause outbreaks in Europe, East Africa, and South Africa [19]. The NDV within genotypes V, VI, and VII are malignant viruses that have recently spread in several regions of the world [20]. The NDV genotypes VII and VIII were reported to have spread in Asia, South Africa, and Europe in the 1990s. Until now, the NDV genotype VII was stated as the fourth panzootical NDV that predominantly spreads to domestic poultry in Asia, Africa, and Europe [19,21].

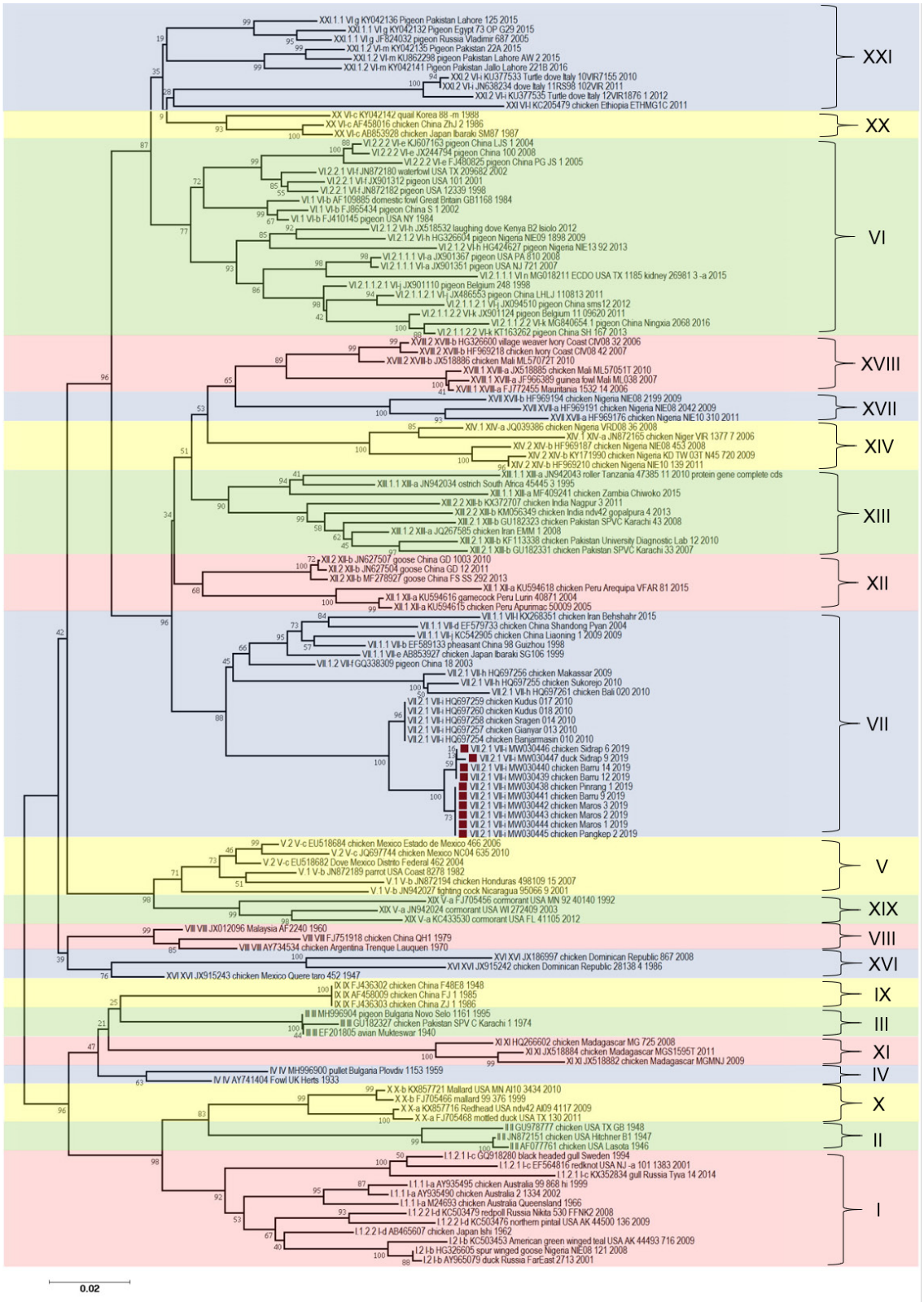

Figure 2. Phylogenetic tree of partial *F* gene of NDV. Indonesian NDV used in this research is shown in the red box. The fusion region from 16 to 153 was analyzed using the MEGA version 7 program. A neighbor-joining bootstrap analysis (1,000 replicates) was carried out using the maximum composite likelihood method.

Table 4. The homology comparison of nucleotide and evolutionary distance with Indonesia ND isolates from the GenBank.

Accession number genbank	Isolat strain		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	Subgenotype
HQ697255	NDV/chicken/ Sukorejo/019/10[a]	1		0.012	0.002	0.093	0.093	0.093	0.093	0.093	0.107	0.107	0.107	0.107	0.107	0.107	0.107	0.107	0.107	0.110	VII.2
HQ697256	NDV/chicken/ Makassar/003/09[a]	2	98.79		0.022	0.093	0.093	0.093	0.093	0.093	0.107	0.107	0.107	0.107	0.107	0.107	0.107	0.107	0.107	0.110	VII.2
HQ697261	NDV/chicken/ Bali/020/10[a]	3	97.76	97.76		0.109	0.109	0.109	0.109	0.109	0.119	0.119	0.119	0.119	0.119	0.119	0.119	0.119	0.119	0.122	VII.2
HQ697254	NDV/chicken/ Banjarmasin/10/10[a]	4	90.70	90.69	89.08		0.000	0.000	0.000	0.000	0.020	0.020	0.020	0.020	0.020	0.020	0.020	0.020	0.020	0.023	VII.2
HQ697257	NDV/chicken/ Gianyar/013/10[a]	5	90.70	90.69	89.08	100		0.000	0.000	0.000	0.020	0.020	0.020	0.020	0.020	0.020	0.020	0.020	0.020	0.023	VII.2
HQ697258	NDV/chicken/ Sragen/014/10[a]	6	90.70	90.69	89.08	100	100		0.000	0.000	0.020	0.020	0.020	0.020	0.020	0.020	0.020	0.020	0.020	0.023	VII.2
HQ697259	NDV/chicken/ Kudus/017/10[a]	7	90.70	90.69	89.08	100	100	100		0.000	0.020	0.020	0.020	0.020	0.020	0.020	0.020	0.020	0.020	0.023	VII.2
HQ697260	NDV/chicken/ Kudus/018/10[a]	8	90.70	90.69	89.08	100	100	100	100		0.020	0.020	0.020	0.020	0.020	0.020	0.020	0.020	0.020	0.023	VII.2
MW030438	Broiler/ Pinrang_1/2019[b]	9	89.27	89.26	88.09	97.96	97.96	97.96	97.96	97.96		0.006	0.006	0.000	0.000	0.000	0.000	0.000	0.006	0.004	VII.2
MW030439	Broiler/ Barru_12/2019[b]	10	89.27	89.26	88.09	97.96	97.96	97.96	97.96	97.96	99.39		0.000	0.006	0.006	0.006	0.006	0.006	0.000	0.002	VII.2
MW030440	Broiler/ Barru_14/2019[b]	11	89.27	89.26	88.09	97.96	97.96	97.96	97.96	97.96	99.39	100		0.006	0.006	0.006	0.006	0.006	0.000	0.002	VII.2
MW030441	Broiler/ Barru_9/2019[b]	12	89.27	89.26	88.09	97.96	97.96	97.96	97.96	97.96	100	99.39	99.39		0.000	0.000	0.000	0.000	0.006	0.004	VII.2
MW030442	Broiler/ Maros_3/2019[b]	13	89.27	89.26	88.09	97.96	97.96	97.96	97.96	97.96	100	99.39	99.39	100		0.000	0.000	0.000	0.006	0.004	VII.2
MW030443	Broiler/ Maros_2/2019[b]	14	89.27	89.26	88.09	97.96	97.96	97.96	97.96	97.96	100	99.39	99.39	100	100		0.000	0.000	0.006	0.004	VII.2
MW030444	Broiler/ Maros_1/2019[b]	15	89.27	89.26	88.09	97.96	97.96	97.96	97.96	97.96	100	99.39	99.39	100	100	100		0.000	0.006	0.004	VII.2
MW030445	Broiler/ Pangkep_2/2019[b]	16	89.27	89.26	88.09	97.96	97.96	97.96	97.96	97.96	100	99.39	99.39	100	100	100	100		0.006	0.004	VII.2
MW030446	Layer/ Sidrap_6/2019[b]	17	89.27	89.26	88.09	97.96	97.96	97.96	97.96	97.96	99.39	100	100	99.39	99.39	99.39	99.39	99.39		0.002	VII.2
MW030447	Itik/Sidrap_9/2019[b]	18	89.02	89.00	87.82	97.75	97.75	97.75	97.75	97.75	99.60	99.80	99.80	99.60	99.60	99.60	99.60	99.60	99.80		VII.2

[a]Strain isolates of ND viruses from Indonesia in Genbank.
[b]Strain isolates of ND viruses 2019 used in this study from South Sulawesi Province, Indonesia.

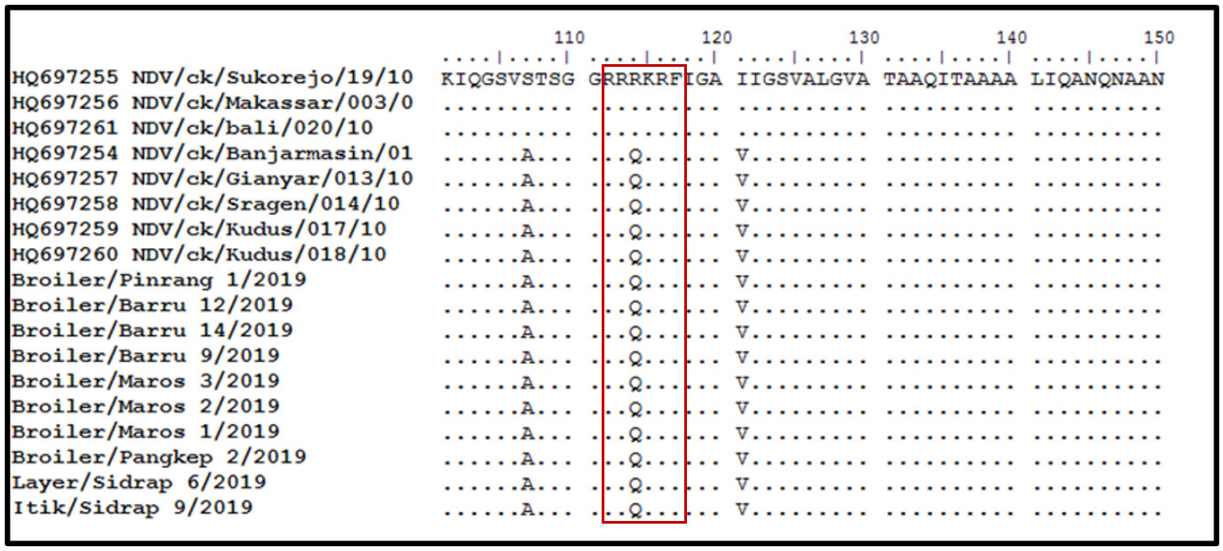

Figure 3. Deduced amino acid sequences in the fusion protein (F0) cleavage site (residue 112 to 117) of NDV isolates from the South Sulawesi Province in 2019 compared with other Indonesian isolates from Genbank.

The genotype VII NDV is classified into three subgenotypes, VII.1.1, VII.1.2, and VII.2 [3]. The VII.1.1 sub-genotype incorporates the former VIIb, VIId, VIIe, VIIj, and VIIl sub-genotypes. Sub-genotype VIIf is called VII.1.2, and sub-genotypes VIIa, VIIh, and VIIi are combined into sub-genotype VII.2. The sub-genotype VII.1.1 originated from China, Malaysia, and Kazakhstan [22,23], and sub-genotypes VII.1.2 and VII.2 were identified from Africa [24]. The ND outbreaks detected in the vaccinated poultry farms indicated that vaccination strategies used in controlling the ND viruses are not effective, so it is necessary to improve the current NDV control strategy. One of the strategies that can be used is developing NDV vaccine seeds according to the NDV circulating in the field. This research can be one of the references for determining the seed vaccine of NDV.

Conclusion

Based on the isolation result and molecular characterization of NDV field isolate from South Sulawesi Province in 2019, the field isolates are included in the NDV sub-genotype VII.2 with 87.82%–97.96% similarity comparing with NDV sub-genotype VII.2 isolates from Indonesia. All isolates had a polybasic F cleavage site motif ^{112}R-R-Q-K-R-F^{117}, suggestive of virulent pathotype of NDV.

List of Abbreviations

F, Fusion protein; HA, Hemagglutination; NDV, ND virus; RNA, Ribonucleic acid; RT-PCR, Reverse transcription-polymerase chain reaction.

Acknowledgments

This research was supported by the PMDSU Research Grant scheme year 2019, Ministry of Research and Innovation Agency of Republic Indonesia.

Conflict of interest

All authors have no conflict of interest in all steps of this study.

Authors' contribution

MES designed the study, collection of data, interpreted the data, and drafted the manuscript. RDS and ONP were involved in designing the study, analyzing the data, and contributed to manuscript preparation.

References

[1] Ashraf A, Shah MS. Newcastle disease: present status and future challenges for developing countries. Afr J Microbiol Res 2014; 8(5):41–416; https://doi.org/10.5897/AJMR2013.6540

[2] Rehan M, Aslam A, Khan MR, Abid M, Hussain S, Amber J, et al. Potential economic impact of Newcastle disease virus isolated from wild birds on commercial poultry industry of Pakistan: a review. Host Viruses 2019; 6(1):1–15; https://doi.org/10.17582/journal.hv/2019/6.1.1.15

[3] Dimitrov KM, Abolnik C, Afonso CL, Albina E, Bahl J, Berg M, et al. Updated unified phylogenetic classification system and revised nomenclature for Newcastle disease virus. Infect Genet Evol 2019; 74:1–15; https://doi.org/10.1016/j.meegid.2019.103917

[4] Li SY, You GJ, Du LJ, Li WW, Liu YY, Du JT, et al. Complete genome sequence of a class I Newcastle disease virus strain isolate from a breeding chicken flock in Sichuan, China. Microbiol Resour Announc. 2019; 8(18):e00337–419; https://doi.org/10.1128/mra

[5] Amarasinghe GK, Ayllón MA, Bào Y, Basler CF, Bavari S, Blasdell KR, et al. Taxonomy of the order Mononegavirales: update 2019. Arch Virol 2019; 164(7):1967–80. https://doi.org/10.1007/s00705-019-04247-4

[6] Maes P, Amarasinghe GK, Ayllón MA, Basler CF, Bavari S, Blasdell KR, et al. Taxonomy of the order mononegavirales: second update 2018. Arch Virol 2019; 164(4):1233–44; https://doi.org/10.1007/s00705-018-04126-4

[7] Alexander DJ. Newcastle disease in the European Union 2000 to 2009. Avian Pathol 2011; 40(6):547–58; https://doi.org/10.1080/03079457.2011.618823

[8] Oberdorfer A, Werner O. Newcastle disease virus: detection and characterization by PCR of recent German isolates differing in pathogenicity. Avian Pathol 1998; 27(3):237–43; https://doi.org/10.1080/03079459808419330

[9] OIE. OIE terrestrial manual Chapter 3.3.4.Ed. Office International Des Epizooties, Paris, France, 2018.

[10] Dharmayanti NLPI, Hartawan R, Hewajuli DA, Indriani R. Phylogenetic analysis of genotype VII of Newcastle disease virus in Indonesia. Afr J Microbiol Res 2014; 8(13):1368–74; https://doi.org/10.5897/AJMR2014.6601

[11] Putri DD, Handharyany E, Soejoedono RD, Setiyono A, Mayasari NLPI, Poetri ON. Genotype characterization of Newcastle Disease virus isolated from commercial chicken flocks in West Java, Indonesia. Pak Vet J 2018; 38 (2):184–8; https://doi.org/10.29261/pakvetj/2018.041

[12] Sarah SI, Nugroho CMH, Kurnia RS, Wardhana DK, Puspitasari H. Phylogenetic analysis and virulence-coding genes characterization of Newcastle disease virus isolated from laying hen brain with torticollis symptom. J Kedokteran Hewan 2019; 13(3): 67–72; https://doi.org/10.21157/j.ked.hewan.v13i3.14493

[13] Wulanjati MP, Wijayanti N, Haryanto A. Phylogenetic analysis of Newcastle disease virus from Indonesian isolates based on DNA-sequence of fusion protein-encoding gene. Biotechnology 2018; 17(2):69–74; https://doi.org/10.3923/biotech.2018.69.74

[14] Radwan MM, Darwish SF, El-Sabagh IM, El-Sanousi AA, Shalaby MA. Isolation and molecular characterization of Newcastle disease virus genotypes II and VIId in Egypt between 2011 and 2012. Virus Genes 2013; 47:311–6; https://doi.org/10.1007/s11262-013-0950-y

[15] Kumar S, Stecher G, Tamura K. MEGA 7: molecular evolutionary genetics analysis version 7.0 for bigger datasets. Mol Evol Genet Anal 2016; 33(7):1870–4; https://doi.org/10.1093/molbev/msw054

[16] Wajid A, Dimitrov KM, Wasim M, Rehmani SF, Basharat A, Bibi T, et al. Repeated isolation of virulent Newcastle disease viruses in poultry and captive non-poultry avian species in Pakistan from 2011–2016. Prev Vet Med 2017; 142:1–6; https://doi.org/10.1016/j.prevetmed.2017.04.010

[17] Putri DD, Handharyany E, Soejoedono RD, Setiyono A, Mayasari NLPI, Poetri ON. Pathotypic characterization of Newcastle disease virus isolated from vaccinated chicken in West Java, Indonesia. Vet World 2017; 10(11):438–44; https://doi.org/10.14202/vetworld.2017.438-444

[18] Alazawy AK, Al Ajeeli KS. Isolation and molecular identification of wild Newcastle disease virus isolated from broiler farms of Diyala Province, Iraq. Vet World 2020; 13(1):33–9; https://doi.org/10.14202/vetworld.2020.33-39

[19] Diel DG, da Silva LHA, Liu H, Wang Z, Miller PJ, Afonso CL. Genetic diversity of *Avian paramyxovirus* type 1: proposal for a unified nomenclature and classification system of Newcastle disease virus genotypes. Infect Genet Evol 2012; 12:1770–9; https://doi.org/10.1016/j.meegid.2012.07.012

[20] Czegledi A, Ujvari D, Somogyi E, et al., Third genome size category of *Avian paramyxovirus* serotype-1 (Newcastle disease virus) and evolutionary implications. Virus Res 2006; 120:36–48; https://doi.org/10.1016/j.virusres.2005.11.009

[21] Miller PJ, Haddas R, Simanov L, Lublin A, Rehmani SF, Wajid A. Identification of new sub-genotype of virulent Newcastle disease virus with potentianpanzootic feature. Infect Genet Evol, 2015; 29:216–29; https://doi.org/10.1016/j.meegid.2014.10.032

[22] Wang Z, Liu H, Xu J, Bao J, Zheng D, Sun C, et al. Genotyping of Newcastle disease viruses isolated from 2002 to 2004 in China. Ann N Y Acad Sci, 2006; 1081:228–39; https://doi.org/10.1196/annals.1373.027

[23] Ebrahimi MM, Shahsavandi S, Moazenijula G, Shamsara M. Phylogeny and evolution of Newcastle disease virus genotypes isolated in Asia during 2008–2011. Virus Genes 2012; 45(1):63–68; https://doi.org/10.1007/s11262-012-0738-5

[24] Snoeck CJ, Ducatez MF, Owoade AA, Olufemi OF, Alkali BR, Tahita MC. Newcastle disease virus in West Africa: new virulent strains identified in non-commercial farms. Arch Virol 2009; 154:47–54; https://doi.org/10.1007/s00705-008-0269-5