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

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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 \( \text{R-R-Q-K-R-F} \) 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.

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
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 inoculum 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 inoculum 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. MW030439); Broiler/Barru 14/2019 (Accession No. MW030440); Broiler/Barru 9/2019 (Accession No. MW030441); Broiler/Maros3/2019 (Accession No. MW030442); Broiler/Maros3/2019 (Accession No. MW030442).
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          | Torticollis, respiratory disorder, paralysed|
| Layer/Barru 7/2019 | Chicken | 21 weeks| Barru    | ≤1,000          | Torticollis, 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'-ATGGGCTCCAGACCCTACCA-3' | 47–67 | 535 |
| Fus-535R | | 5'-CTGCCACCTGACATTGCATGAAAT-3' | 557–81 | |

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

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)   | positive        |
| 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).
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 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.
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 | Isolat strain | 1   | 2   | 3   | 4   | 5   | 6   | 7   | 8   | 9   | 10  | 11  | 12  | 13  | 14  | 15  | 16  | 17  | 18  | Subgenotype |
|------------------|----------------|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----------|
| HQ697255         | NDV/chicken/   | 1.012 | 0.002 | 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 | 0.107 | 0.107 | VII.2  |
| Makassar/003/09  |                | 2.9879 | 0.022 | 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 | 0.107 | 0.107 | VII.2  |
| Bal/020/10      |                | 3.97.76 | 97.76 | 0.109 | 0.109 | 0.109 | 0.109 | 0.109 | 0.111 | 0.111 | 0.112 | 0.111 | 0.111 | 0.111 | 0.111 | 0.111 | 0.111 | 0.111 | VII.2  |
| Saputri          |                | 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 | 0.020 | VII.2  |
| MW030438         | Broiler/       | 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 | 0.020 | VII.2  |
| Pinrang_1/2019^b|                | 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 | 0.020 | VII.2  |
| MW030439         | Broiler/       | 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 | 0.020 | VII.2  |
| Barru_12/2019^b |                | 8.90.70 | 90.69 | 89.08 | 100  | 100  | 100  | 0.020 | 0.020 | 0.020 | 0.020 | 0.020 | 0.020 | 0.020 | 0.020 | 0.020 | 0.020 | 0.020 | VII.2  |
| Broiler/         |                | 9.90.70 | 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 | 0.020 | VII.2  |
| MW030440         | Broiler/       | 10.90.70 | 90.69 | 89.08 | 97.96 | 97.96 | 97.96 | 97.96 | 97.96 | 97.96 | 97.96 | 97.96 | 97.96 | 97.96 | 97.96 | 97.96 | 97.96 | 97.96 | VII.2  |
| Barru_14/2019^b |                | 11.90.70 | 90.69 | 89.08 | 97.96 | 97.96 | 97.96 | 97.96 | 97.96 | 97.96 | 97.96 | 97.96 | 97.96 | 97.96 | 97.96 | 97.96 | 97.96 | 97.96 | VII.2  |
| Broiler/         |                | 12.90.70 | 90.69 | 89.08 | 97.96 | 97.96 | 97.96 | 97.96 | 97.96 | 97.96 | 97.96 | 97.96 | 97.96 | 97.96 | 97.96 | 97.96 | 97.96 | 97.96 | VII.2  |
| MW030441         | Maros_3/2019^b | 13.90.70 | 90.69 | 89.08 | 97.96 | 97.96 | 97.96 | 97.96 | 97.96 | 97.96 | 97.96 | 97.96 | 97.96 | 97.96 | 97.96 | 97.96 | 97.96 | 97.96 | VII.2  |
| Broiler/         |                | 14.90.70 | 90.69 | 89.08 | 97.96 | 97.96 | 97.96 | 97.96 | 97.96 | 97.96 | 97.96 | 97.96 | 97.96 | 97.96 | 97.96 | 97.96 | 97.96 | 97.96 | VII.2  |
| MW030443         | Maros_2/2019^b | 15.90.70 | 90.69 | 89.08 | 97.96 | 97.96 | 97.96 | 97.96 | 97.96 | 97.96 | 97.96 | 97.96 | 97.96 | 97.96 | 97.96 | 97.96 | 97.96 | 97.96 | VII.2  |
| Broiler/         |                | 16.90.70 | 90.69 | 89.08 | 97.96 | 97.96 | 97.96 | 97.96 | 97.96 | 97.96 | 97.96 | 97.96 | 97.96 | 97.96 | 97.96 | 97.96 | 97.96 | 97.96 | VII.2  |
| MW030444         | Maros_1/2019^b | 17.90.70 | 90.69 | 89.08 | 97.96 | 97.96 | 97.96 | 97.96 | 97.96 | 97.96 | 97.96 | 97.96 | 97.96 | 97.96 | 97.96 | 97.96 | 97.96 | 97.96 | VII.2  |
| Broiler/         |                | 18.90.70 | 90.69 | 89.08 | 97.96 | 97.96 | 97.96 | 97.96 | 97.96 | 97.96 | 97.96 | 97.96 | 97.96 | 97.96 | 97.96 | 97.96 | 97.96 | 97.96 | VII.2  |

^aStrain isolates of ND viruses from Indonesia in Genbank.
^bStrain isolates of ND viruses 2019 used in this study from South Sulawesi Province, Indonesia.
The genotype VII NDV is classified into three sub-genotypes, VII.1.1, VII.1.2, and VII.2 [3]. The VII.1.1 sub-genotype incorporates the former VIIb, VIId, VIIe, VIIj, and VIII 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 112R-R-Q-K-R-F117, 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.

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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.

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