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Newcastle Disease Virus on East Java Isolate based on Fusion Protein: An Epidemiology Study.

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ABSTRACT

This research was to study the demographic transmission of ND virus (NDV) in some areas of East Java area, Indonesia, based on fusion protein. This research was an observational and cross sectional study aimed to obtain NDV isolate from some chicken poultry breeders which were suspected with ND. ND diagnosis was determined based on: clinical signs, gross pathology, the time of the death of embryo when inoculated in the embryonated eggs, hemagglutination (HA) test, hemaglutination-inhbition (HI) test, and fusion (F) protein. From this study, there were 58 isolates suspected with NDV (based on clinical sign and gross pathology) from 58 different poultry breedersand 15districts in East Java. From the suspected 58 NDV isolates, only one isolate which has negative HA test and 19 with negative HI test, while the remaining 39 isolates were NDV positive.All isolates were purified by the F protein and by using a pair of AA-HL primer (362bp nucleotides) and it resulted in20 NDV isolates whichpossess F protein gene, with the code of tpM3, ktM1,and trM6 (Malang), kdT1 (Tulungagung, kdB4 and kdB6 (Blitar), pwS3 and pwS4 (Surabaya), bjMK2, bjMK3, and bjMK5 (Mojokerto), ppM1 and ppM2 (Madiun), pkP1 (Ponorogo), psN1, psN2, and psN3 (Nganjuk), ppK1 and ppK3 (Kediri) pkB5 (Bojonegoro). All these isolates are classified in velogenic strain. Based on the nucleotide basis of the F protein gene,94-99% between East Java isolates is homological to Banjarmasin, Sukorejo, Gianyar (Bali Province), Sragen and Kudus isolates, but not to other East Java isolates.

Keywords: Newcastle Disease Virus, F protein

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INTRODUCTION

Newcastle disease (ND) is a contagious poultry disease affecting many domestic and wild avian species, causing great impact for global trade. It is one of zoonosis which can cause human mild conjunctivitis and influenza-like symptoms, caused by virulent strains of avian paramyxovirus type 1 (APMV-1) serotype of the Avulavirus genus belonging to the Paramyxovirinae subfamily, Paramyxoviridae family. NDV strains can be categorised into three strains, namely, velogenic (highly virulent), mesogenic (intermediate virulence) or lentogenic (nonvirulent). ND endemic in Indonesia is caused by the most virulence velogenic strain. Velogenic is a very contagious strain, it produces severe nervous and respiratory signs, spreads rapidly, and causes up to 90% mortality. On the other hand, mesogenic strain may cause coughing, affect egg quality and production. It also has a potential to cause 10% of mortality. Whereas, lentogenic strain has low virulence, produces mild signs with negligible mortality rates.

Newcastle disease virus spreads primarily through direct contact between healthy birds and infected birds. The disease is transmitted through infected birds' droppings and secretions from nose, mouth, and eyes. NDV spreads rapidly among birds which are kept in confinement, like thecommercially raised chickens. Virulent NDV strains are endemic in poultry in most of Asia, Africa, and some countries of North and South America. Meanwhile, other countries including USA and Canada are free of those strains and they could maintain that status with import restrictions and eradication by destroying the infected poultry[1].

During replication, APMV-1 particles are produced with a precursor glycoprotein;a F0 has to be cleaved to F1 and F2 to become infectious virus particles. This post-translational cleavage is mediated by host-cell proteases. Trypsin is capable of cleaving F0 for all NDV strains[2]. Most of specific NDV molecular is coded by F gene at variable region, it is important for signal peptide [amino acid (aa)], cleavage activation sequence (aa 112–116), fusion hydrophobic region (aa 117–142), and signed by variable and conserved regions. Nucleotide sequence fragment of F-gene is the standard criteria of genotyping and pathogenicity molecular of NDV [2,3]. F-gene function is a fusion factor to host cells. Without the gene, the NDV cannot infect the host cell.

The virus toxication is determined by the pattern of ^{112}R/K-R-Q-K/R-R^{116} at C-terminus F2 protein and F (phenylalanine) at N-terminus F1 protein (residue 117). The history and management of a poultry breeder can be used as the supportive data for the determination of source NDV. Japanese scientists found different sequence of cleavage site in F gene between isolated lentogenic strain vaccine and wild virus NDV; they have proved that ^{112}R-R-Q-K-R-F^{117} motive belongs to velogenic strain. The result has been confirmed by MDT (Mean Death Time) and ICPI (Intracerebral Pathogenicity Index)[3]. Some ND vaccination programs in Indonesia failed and the failure may be caused by inappropriate antigenic structure between the vaccine and wild isolate. The study was NDV epidemiological study in East Java as the centre of poultry breeders where ND is still a major problem. This study aims at: 1) making an isolation and identification of NDV wild isolate from ND cases in some districts of East Java; 2) performing an isolation of F protein; 3) identifying F protein gene; 4) conducting a phylogeny study base of F protein gene. From the first study, there will be a continuation of F protein immunogenicity detection as a basis to develop wild isolate Indonesian seed vaccine.

MATERIALS AND METHODS

This study was done based on the case reports at some poultry breeders which were suspected to suffer from ND. It was carried out in 15 cities/regencies of East Java Province which had the number of chickens more than 50,000, namely, Blitar, Malang, Tulungagung, Sidoarjo, Surabaya, Mojokerto, Ngawi, Madiun, Ponorogo, Jombang, Nganjuk, Kediri, Bojonegoro, Tuban, and Kertosono. This study was started in February 26th, 2015. The number of samples used in this research were 336 chickens taken through random sampling method from wet markets, local poultry breeders and veterinarians who had chickens with NDV symptoms. The ND virus was determined by anamnesis, clinical signs, gross pathologic, embryo death time (in embryonated egg), HA test and HI test. The viruses were inoculated in allantois fluid of 9-11 days old embryonated eggs. Then, the death time and growth the embryos after inoculation were recorded. After that, their ability to agglutinate sheep erythrocytes were tested to calculate virus titre. HI test was done using specific antibody[4]. The work was done aseptically using biohazard laminar airflow level II.

Molecular analysis of Newcastle disease virus field isolate

Molecular analysis of the ND virus was performed by using HI test against ND virus specific antibodies. Extraction of the RNA was performed using the QIAamp Viral RNA Kit (QIAGEN) method based on the recommended standard procedures, using a allantoic fluid test material containing the ND virus. The extraction result of each sample was then used as a template for the PCR amplification process. The DNA amplification process was carried out using *OneStep PCR Primer* method, with a pair of F gene primer *(primer AAHL sense dan AAHL antisense*).

OneStep RT-PCR amplification stage was started from a reverse transcriptation cycle at a temperature of 48 ° C for 30 minutes, followed by an initial denaturation cycle at a temperature of 95 ° C for 15 minutes. The PCR amplification stage consisted of 30 cycles and three stages, beginning with a denaturation stage at a temperature of 94 ° C for 30 seconds, followed by annealing stage at a temperature of 53 ° C for one minute, and the last stage was the extension at a temperature of 72 ° C for one minute. The next stage was the final extension at a temperature of 72 ° C for 10 minutes and ended with the cooling process (hold) at a temperature of 4 ° C. The product result of Simplex OneStep RT-PCR then stored in a freezing Temperature of minus 20° C, to be separated through electrophoresis in agarose gel[5].

The next stage after electrophoresis was a nucleotide sequencing of F gene of ND virus. Then, the nucleotide sequence of the F gene which had been obtained was anlyzed using homology and phylogenetic analysis. The phylogenetic analysis was carried out by collecting data on the sequencing of ND virus genetic Group I to VIII from NCBI/ Gene Bank as well as data on the sequencing of ND virus of chickens isolates in East Java before processed using CLC Sequenser Viewer 6.7 software.

Identification of Protein F of ND Virus at Field Isolate

The protein of ND virus in the Allantoic fluid was analysed using SDS-PAGE test. A total allantoic fluid of 5µl was put in 12% separation gel before it was electrophoresed at room temperature with a voltage of 200V. After that, the gel was stained using 0.1% commasie blue solution for 24 hours and it was continued with destaining process using methanol and glacial acetic acid for about 3 hours. The profile of protein F of ND virus was tested using western blothing to determine the anti genity of the protein F of the ND virus which was reacted with a specific polyclonal antibody of ND. The process was preceded by SDS-PAGE test but the electrophoresis stage was not followed by a staining. The gels were transferred onto nitrocellulose membranes. After being transferred to the membrane, blocking for 24 hours was carried out, followed by washing, ND antibody addition, anti-chicken conjugate addition and TMB substrate addition until the colour changes reaction happened. Then, the reaction was ended using destilated water and the results were analyzed.

The Isolation of Protein F of ND Virus with Electro Elution

Protein gel had been electrophoresed (such as the SDS-PAGE sequence above) had a specific molecular weight. Protein F as the isolation target was taken by cutting the gel. Gel pieces then put into a selofan bag and added with 1 ml of PBS before the two selofan ends were tied. Put the selofan containing protein F band in the electroelution chamber with a temperature of 4ºC containing E-buffer solution. The electric current used was 100 volts, 50 mA and 12 W. The electroelution time was 4 hours before the protein was collected in an eppendorf tube. After the isolation of protein F by electroelution was carried out, the protein content was then determined using Quant-iT Kit, and the results were verified by Qubit TM Fluorometer. The purification process of the protein F obtained was then conducted by using dialysis method. After the purification process ended, adjuvant addition process was made. Oil emulsion adjuvant of Montanide ISA 70 was added to protein F as the result of purification process with a ratio of 70:30 (adjuvant: protein F).

RESULTS AND DISCUSSION

The anamnesis and clinical signs are referred to ND, such as the death of some chickens, chicken weakness, dropped egg production, torticollis, nasal serous discharge, greenish diarrhoea, and inflammation around eye. Macroscopic lesion includes petechial of tracheal, intestine and proventriculus, haemorrhage of

brain and meninges, enlarged of lien and liver, and the swelling around eye. From this study, it was found around 58 isolates which are suspected NDV from 58 poultry breeders.

Some microorganisms have hemaglutinin, such as myxo virus group (*influenza virus, parainfluenza virus, mumps virus, morbilivurs, NDV, respiratory syncial virus), rubeola virus, adenovirus, reovirus, Staphylococcus sp., Vibrio sp.*, *Hemophilus influenza, Salmonella sp., E. coli, Clostridium botulinum, Pseudomonas sp*. Hemaglutinin is a surface protein marker that agglutinated particular erythrocyte.The presence of hemaglutinin was tested by using Hemagglutinantion (HA) test. From 58 isolates, just one isolate that has negative HA test.

One of the conventional methods to determine the pathotype of the virus is by virus inoculation in that the mean of death time is calculated. It was found that velogenic strain killed the embryo at less than 60 hours,mesogenic strain at 60-90 hours, and lentogenic strain by more than 90 hours[6,7]. In this work, some isolates show that the embryos died before 60 hours with abnormal growth; these characteristics are belong to velogenic strain. Yet, some of the death may be caused by bacterial infection since the allantoic fluid is cloudy. The HI test was used to assess the specific antibody. It was shown by 39 positive isolates.

The 39 isolates were detected by the F protein gene with PCR method and a pair of AA-HL primer (362 bp nucleotides). It resulted in 20 isolates where the F protein was amplified (Figure 1-6). The nucleotide basis of F protein will be sequenced for phylogenicity study and used as a tool for ND vaccine seed development.

The severity of the disease was influenced by the NDV strain.Velogenic strain is the most virulent and contagious strain, while mesogenic strain is the intermediate virulent strain, and the non-virulent strain is the lentogenic strain. Velogenic strain causes severe neurologic and respiratory issue with high mortality up to 90%.Mesogenic strain was marked by cough and decreased egg quality and production with 10% of mortality level. While lentogenic strain just marked by mild clinical signs and rare animal death. Acute case of sudden animal death is particularly caused by velogenic strain[1,8], respiratory signs followed by depression, greenish watery diarrhoea, head and neck inflammation, neurologic signs (torticollis, ataxia, tremor) are included in the characteristic of ND which was infected by the most virulent virus which is viscerotrophicvelogenic strain, and it often suffered by the vaccinated poultry breeder. Macroscopic lesions were just detected in viscerotrophicvelogenic ND. So that, it can be concluded that the 20 NDV isolates in this research are a viscerotrophicvelogenic strain.

The F protein genes were sequenced (see List 1) and analysed. From the nucleotide bases and the phylogeny tree, it can be seen that the 20 isolates have such close relation to the isolates of Bojonegoro, Sukorejo, Gianyar (Bali Province), Sragen and Kudus, but not to East Java isolates.

Figure 1. The primary pair, AA-HL 362 bpnucleotides, was able to amplify the ktM1, tpM3, ktM1, tpM3, trM6, trM6, and kdt1 samples

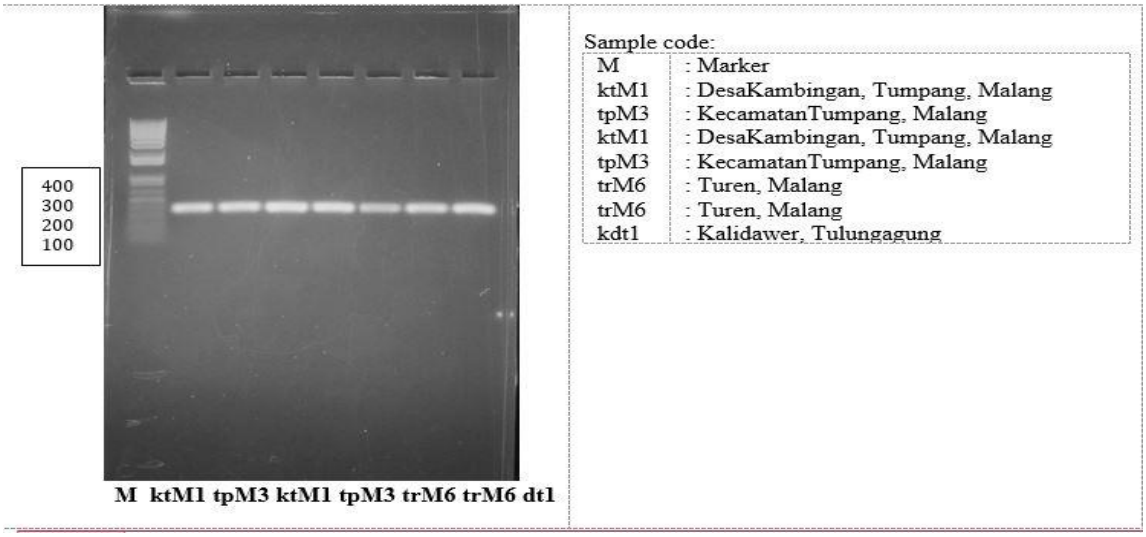

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Figure 2. The primary pair, AA-HL 362 bpnucleotides, was able to amplify the kdb4, kdB6, pwS3 and pwS4 samples, but was not able to amplify the pwS1, pdB8, pdB14, pdB16, and pdB17 samples

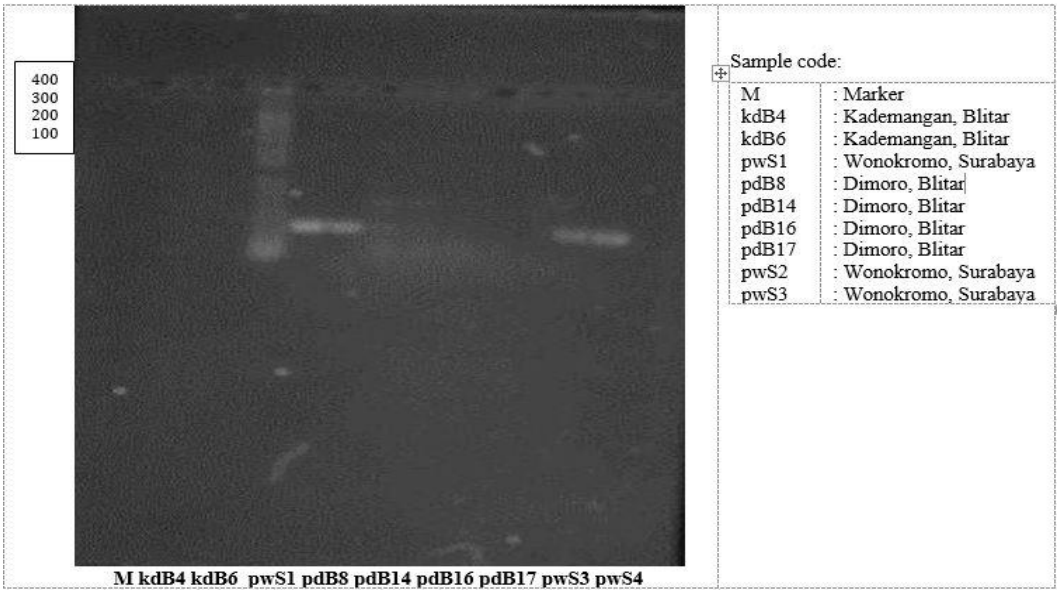

Figure 3. The primary pair, AA-HL 362 bpnucleotides, was able to amplify the bjMK3, pkP2, bjMK5, ppM1 samples, but was not able to amplify the pJ2 and sjJ1 samples

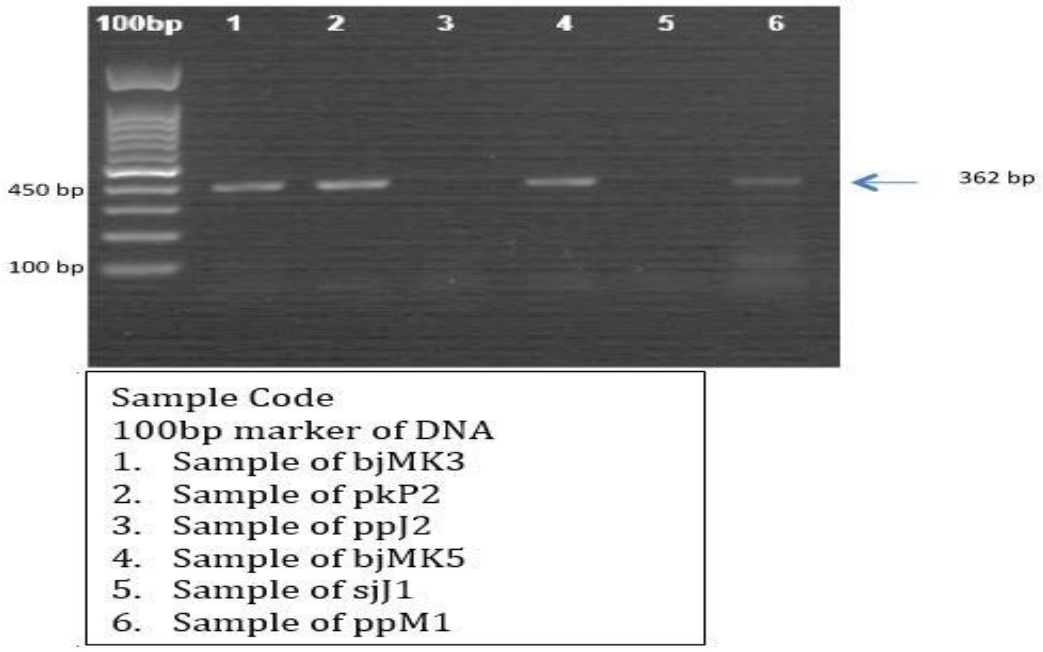

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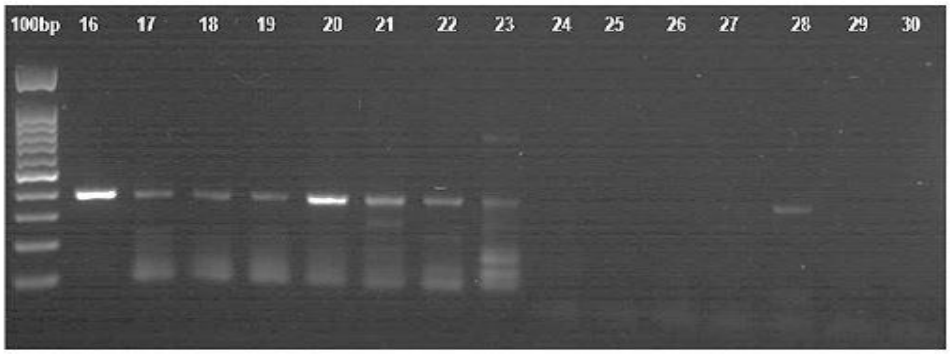
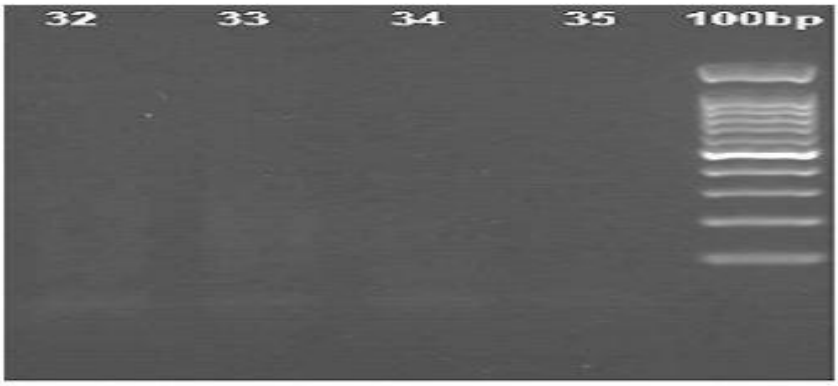
Figure 4. The primary pair, AA-HL 362 bp nucleotides, was able to amplify the pkP1, psN1, ppM2, psN3, ppK1, ppK3, psN2, bjMK2, pkB5 samples, but was not able to amplify the pkB1, pkB2, pkB3, pkB4, pkB6, mkT1 samples



Sample Code

100bp marker of DNA
16. Sample of pkP1
17. Sample of psN1
18. Sample of ppM2
19. Sample of psN3
20. Sample of ppK1
21. Sample of ppK3
22. Sample of psN2
23. Sample of bjMK2
24. Sample of pkB1
25. Sample of pkB2
26. Sample of pkB3
27. Sample of pkB4
28. Sample of pkB5
29. Sample of pkB6
30. Sample of mkT1

Figure 5. The primary pair, AA-HL 362 bp nucleotides, could not amplify the mkT2, mkT3, sjJ2 and sjJ3 samples



Sample Code

100bp marker of DNA
32. Sample of mkT2
33. Sample of mkT3
34. Sample of sjJ2
35. Sample of sjJ3

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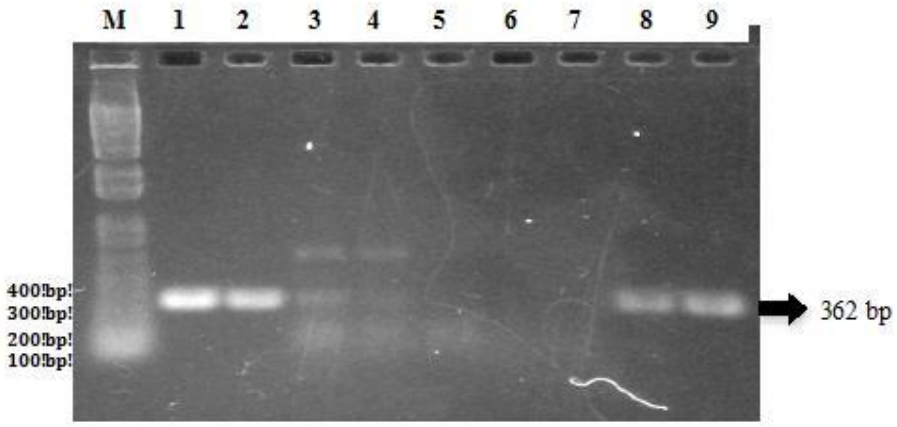
Figure 6. The primary pair, AA-HL 362 bp nucleotides, were able to amplify the kdB4, kdB10, pdB8, pdB14, but not to pwS1, pwS8, pdB9, pdB12, pdB15 samples



M : Marker
1 : code kdB4 (positive)
2 : code kdB10 (positive)
3 : code pwS1
4 : code pdB8
5 : code pdB9
6 : code pdB12
7 : code pdB15
8 : code pdB8 (positive)
9 : code pdB14 (positive)

List 1. Result of F protein genes

Code ktM 1 (sample 1)

TTGGAGGATGTTGGCAGCATTTTGGTTGGCTTGTATTAAGGCCGCAGCTGCTGTTATCTGTGCCGCTGTCGCAACCCCGAG
AGCTACACTGCCAATAACGGCACCTATAAAGCGTTTCTGTCTCCTTCCTCCGGACGTGGCCACCGACCCTTGGATCTTACG
GATGGAATCGCCAAGGGGAGTGAGCAAGGTAGTCAGTGTTCTGTTGTATGCCTCTAATGGGGCTTTTGCACACGCCTCTT
TATCCTTAGGCATATTCGGGAGCAACTTGACTATGATTGACCCTGTCTGAGATGAGGTGTATACATTGACTGCCTTATCTCC
TGTTACGACAATCCCTKCAGCTGCAAGAGGCCTGCCATCAAAA

Code tpM 3 (sample 2)

TTTTGGAGGATGTTGGCAGCATTTTGGTTGGCTTGTATTWAGGCCGCAGCTGCTGTTATCTGTGCCGCTGTCGCAACCCC
GAGAGCTACACTGCCAATAACGGCACCTATAAAGCGTTTCTGTCTCCTTCCTCCGGACGTGGCCACCGACCCTTGGATCTT
ACGGATGGAATCGCCAAGGGGAGTGAGCAAGGTAGTCAGTGTTCTGTTGTATGCCTCTAATGGGGCTTTTGCACACGCCT
CTTTATCCTTAGGCATATTCGGGAGCAACTTGACTATGATTGACCCTGTCTGAGATGAGGTGTATACATTGACTGCCTTATC
TYCTGTTACGACAATCCCTGCAGCTGCAAGAGGCCTGCCATCAAAA

Code ktM 1 (sample 3)

TTGGAGGATGTTGGCAGCATTTTGGTTGGCTTGTATTAAGGCCGCAGCTGCTGTTATCTGTGCCGCTGTCGCAACCCCGAG
AGCTACACTGCCAATAACGGCACCTATAAAGCGTTTCTGTCTCCTTCCTCCGGACGTGGCCACCGACCCTTGGATCTTACG
GATGGAATCGCCAAGGGGAGTGAGCAAGGTAGTCAGTGTTCTGTTGTATGCCTCTAATGGGGCTTTTGCACACGCCTCTT
TATCCTTAGGCATATTCGGGAGCAACTTGACTATGATTGACCCTGTCTGAGATGAGGTGTATACATTGACTGCCTTATCTCC
TGTTACGACAATCCCTKCAGCTGCAAGAGGCCTGCCATCAAAA

Code tpM3 (sample 4)

TTTTGGAGGATGTTGGCAGCATTTTGGTTGGCTTGTATTWAGGCCGCAGCTGCTGTTATCTGTGCCGCTGTCGCAACCCC
GAGAGCTACACTGCCAATAACGGCACCTATAAAGCGTTTCTGTCTCCTTCCTCCGGACGTGGCCACCGACCCTTGGATCTT
ACGGATGGAATCGCCAAGGGGAGTGAGCAAGGTAGTCAGTGTTCTGTTGTATGCCTCTAATGGGGCTTTTGCACACGCCT
CTTTATCCTTAGGCATATTCGGGAGCAACTTGACTATGATTGACCCTGTCTGAGATGAGGTGTATACATTGACTGCCTTATC
TYCTGTTACGACAATCCCTGCAGCTGCAAGAGGCCTGCCATCAAAA

Code trM6 (sample 5)

TTGTGGAGGATGTTGGCAGCATTTTGGTTGGCTTGTATTWAGGCCGCAGCTGCTGTTATCTGTGCCGCTGTCGCAACCCC
GAGAGCTACACTGCCAATAACGGCACCTATAAAGCGTTTCTGTCTCCTTCCTCCGGACGTGGCCACCGACCCTTGGATCTT
ACGGATGGAATCGCCAAGGGGAGTGAGCAAGGTAGTCAGTGTTCTGTTGTATGCCTCTAATGGGGCTTTTGCACACGCCT
CTTTATCCTTAGGCATATTCGGGAGCAACTTGACTATGATTGACCCTGTCTGAGATGAGGTGTATACATTGACTGCCTTATC
TYCTGTTACGACAATCCCTGCAGCTGCAAGAGGCCTGCCATCAAAA

Code trM6 (sample 6)

TTGTGGAGGATGTTGGCAGCATTTTGGTTGGCTTGTATTWAGGCCGCAGCTGCTGTTATCTGTGCCGCTGTCGCAACCCC
GAGAGCTACACTGCCAATAACGGCACCTATAAAGCGTTTCTGTCTCCTTCCTCCGGACGTGGCCACCGACCCTTGGATCTT
ACGGATGGAATCGCCAAGGGGAGTGAGCAAGGTAGTCAGTGTTCTGTTGTATGCCTCTAATGGGGCTTTTGCACACGCCT
CTTTATCCTTAGGCATATTCGGGAGCAACTTGACTATGATTGACCCTGTCTGAGATGAGGTGTATACATTGACTGCCTTATC
TYCTGTTACGACAATCCCTGCAGCTGCAAGAGGCCTGCCATCAAA

Code kdt 1 (sample 7)

TTTGGAGGATGTTGGCAGCATTTTGGTTGGCTTGTATTAAGGCCGCAGCTGCTGTTATCTGTGCCGCTGTCGCAACCCCGA
GAGCTACACTGCCAATAACGGCACCTATAAAGCGTTTCTGTCTCCTTCCTCCGGACGTGGCCACCGACCCTTGGATCTTAC
GGATGGAATCGCCAAGGGGAGTGAGCAAGGTAGTCAGTGTTCTGTTGTATGCCTCTAATGGGGCTTTTGCACACGCCTCT
TTATCCTTAGGCATATTCGGGAGCAACTTGACTATGATTGACCCTGTCTGAGATGAGGTGTATACATTGACTGCCTTATCTY
CTGTAYACGACAATCCCTGCAGCTGCAAGAGGCCTGCCATCAAAA

Code kdB 10 (sample 9)

GGGAGGATGTTGGCAGCATTCTGGTTGGCTTGKRTTAGGGCCGCAGCTGCAGTTATCTGTGCCGCTGTTGCAACTCCGAG
AGCGACACTGCCAATGATGGCACCTATAAAGCGTTTCCGTCTCCTTCCTCCAGACGTGGACACGGACCCTTGGATCTTGCG
GATGGAATCGCCGAGGGGGGTGAGCAATGTAGTCAGTGTCCTATTATATGCTTCCAACGGGGCTCTTGCACACGCCTCTT
TATCCTTGGGCATATTCGGGAGCAACTTGACTATGATTGACCCAGTTTGAGACGAGGTGTAGATATTGACTGCTGCTYGW
CGTCSTGTWACAGKAMRATCCCTGCAGCCGCAAGAGGCTTGCCATCAAAA

Code BJMK_2_ND

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AGCTACACTGCCAATAACGGCACCTATAAAGCGTTTCTGTCTCCTTCCTCCGGACGTGGCCACCGACCCTTGGATCTTACG
GATGGAATCGCCAAGGGGAGTGAGCAAGGTAGTCAGTGTTCTGTTGTATGCCTCTAATGGGGCTTTTGCACACGCCTCTT
TATCYTTAGGCATATTCRGGAGCAACTTGACTATGATTGATCCCTGTYTGAGATGAGGTGTATACATTGACTGCSYTATCTS
CWGTTACGACAAYCCYTKCAGCTGCAAGAGGCTTGCCATCAAA

Code BJMK3_ND

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GATYTTACCGMTGGAATCKCCAASGGGAGKGAKCAAKGTASTCAKTGTWSTGTKGKATKCYTYTAATGGGGCTTTTGCWC
AMSCCTCTTTATCYTTRGGCATATTCMGGAGCAACTTGAYTATGATKGAYCCTGTCKGARATRAAGTGWATACATTGACT
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BJMK_5_ND

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TACGGATGGAATCGCCAAGGGGAGTGAGCAAGGTAGTCAGTGTTCTGTTGTATGCCTCTAATGGGGCTTTTGCACACGCC
TCTTTATCCTTAGGCATATTCGGGAGCAACTTGACTATGATTGACCCTGTCTGAGATGAGGTGTATACATTGACTGCCTTAT
CTCCTGTATRCGACAATCCCTGCAGCTGCAAGAGGCCTTGCCATCAAA

PKB_5_ND

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GGATGGAATCGCCAAGGGGAGTGAGCAAGGTAGTCAGTGTTCTGTTGTATGCCTCTAATGGGGCTTTTGCACACGCCTCT
TTATCCTTAGGCATATTCGGGAGCAACTTGACTATGATTGACCCTGTCTGAGATGAGGTGTATACATTGACTGCCTTWTCT
SYWSTTACTACWWTCCCTGCAGCTGCAAGAGGCCTGCCATCAAAA

PKP_1_ND

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GGATGGAATCGCCAAGGGGAGTGAGCAAGGTAGTCAGTGTTCTGTTGTATGCCTCTAATGGGGCTTTCGCACACGCCCCT
TTATCCTTGGGCATATTCGGGAGCAACTTGACTATGATTGACCCTGTCTGAGATGAGGTGTATACATTGATTGCCTTATCTC
CTGTTACTATAATCCYTGCAGCTGCAAGAGGCCTGCCATCAAAA

PKP_2_ND

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GAGCTACACTGCCAATAACGGCACCTATAAAGCGTTTCTGTCTCCTTCCTCCGGACGTGGCCACCGACCCTTGGATCTTAC
GGATGGAATCGCCAAGGGGAGTGAGCAAGGTAGTCAGTGTTCTGTTGTATGCCTCTAATGGGGCTTTCGCACACGCCTCT
TTATCCTTGGGCATATTCGGGAGCAACTTGACTATGATTGACCCTGTCTGAGATGAGGTGTATACATTGATTGCCTTATCTC
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PPK_1_ND

TTGGAGGATGTTGGCAGCATTTTGGTTGGCTTGTWTTAAGGCCGCAGCTGCTGTTATCTGTGCTGCTGTCGCAACCCCGA
GAGCTACACTGCCAATAACGGCACCTATAAAGCGCTTCTGTCTCCTTCCTCCGGACGTGGCCACCGACCCTTGGATCTTAC
GGATGGAGTCGCCAAGGGGAGTGAGCAAGGTAGTCAGTGTTCTGTTGTATGCCTCTAATGGGGCTTTTGCACACGCCTCT
TTGTCCTTAGGCATATTCGGGAGCAACTTGACTATGATTGACCCTGTCTGAGATGAGGTGTATACATTGACTGCCTTATCTC
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PPK_3_ND

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GGATGGAATCGCCAAGGGGAGTGAGCAAGGTAGTCAGTGTTCTGTTGTATGCCTCTAATGGGGCTTTTGCACACGCCTCT
TTATCCTTAGGCATATTCGGGAGCAACTTGACTATGATTGACCCTGTCTGAGATGAGGTGTATACATTGACTGCCTTMTCK
YMTGTTACGAYAATCCCTGCAGCTGCAAGAGGCTTGCCATCAAAA

PPM_1_ND

GGAGGATGTTGGCAGCATTTTGGTTGGCTTGTRYYAAKGSCGCAGCTGCTGTTATCTGTGCCGCTGTCGCAACCCCGAGA
GCTACACTGCCAATAACGGCACCTATAAAGCGTTTCTGTCTCCTTCCTCCGGACGTGGCCACCGACCCTTGGATCTTACGG
ATGGAATCGCCAAGGGGAGTGAGCAAGGTAGTCAGTGTTCTGTTGTATGCCTCTAATGGGGCTTTTGCACACGCCTCTTT
ATCCTTAGGCATATTCGGGAGCAACTTGACTATGATTGACCCTGTCTGAGATGAGGTGTATACATTGACTGCCTWWTCK
MCKSTTGASSWCRATCYCTGCAGYTGCAAGAGGCTTGGCCATCAAAA

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PPM_2_ND

GGAGGATGTTGGCAGCATTTTGGTTGGCTTGTATTAWGGSCGCAGCTGCTGTTATCTGTGCCGCTGTCGCAACCCCGAGA
GCTACACTGCCAATAACGGCACCTATAAAGCGTTTCTGTCTCCTTCCTCCGGACGTGGCCACCGACCCTTGGATCTTACGG
ATGGAATCGCCAAGGGGAGTGAGCAAGGTAGTCAGTGTTCTGTTGTATGCCTCTAATGGGGCTTTTGCACACGCCTCTTT
ATCCTTAGGCATATTCGGGAGCAACTTGACTATGATTGACCCTGTCTGAGATGAGGTGTATACATTGACTGCCTTATCTCC
WGTTACGACAATCCCTGCAGACTKYAAGAGGCCTGCCATCAAAA

PSN_1_ND

GCGCAGCTGCTGTTATCTGTGCCGCTGTCGCAACCCCGAGAGCTACACTGCCAATAACGGCACCTATAAAGCGTTTCTGTC
TCCTTCCTCCGGACGTGGCCACCGACCCTTGGATCTTACGGATGGAATCGCCAAGGGGAGTGAGCAAGGTAGTCAGTGTT
CTGTTGTATGCCTCTAATGGGGCTTTTGCACACGCCTCTTTATCCTTAGGCATATTCGGGAGCAACTTGACTATGATTGACC
CTGTCTGAGATGAGGTGTATACATTGACTGCCTTATCTCCTGTTACGACAATCCCTGCAGCTGCAAGAGGCCNTGCCATCA
A

PSN_2_ND

TGGAGGATGTTGGCAGCATTTTGGTTGGCTTGTATTWASGCCGCAGCTGCTGTTATCTGTGCCGCTGTCGCAACCCCGAG
AGCTACACTGCCAATAACGGCACCTATAAAGCGTTTCTGTCTCCTTCCTCCGGACGTGGCCACCGACCCTTGGATCTTACG
GATGGAATCGCCAAGGGGAGTGAGCAAGGTAGTCAGTGTTCTGTTGTATGCCTCTAATGGGGCTTTTGCACACGCCTCTT
TATCCTTAGGCATATTCGGGAGCAACTTGACTATGATTGACCCTGTCTGAGATGAGGTGTATACATTGACTGCCTTATCTCC
TGTTACGMCAATCCCTGCAGCTGCAAGAGGCCTGCCATCAAAA

PSN_3_ND

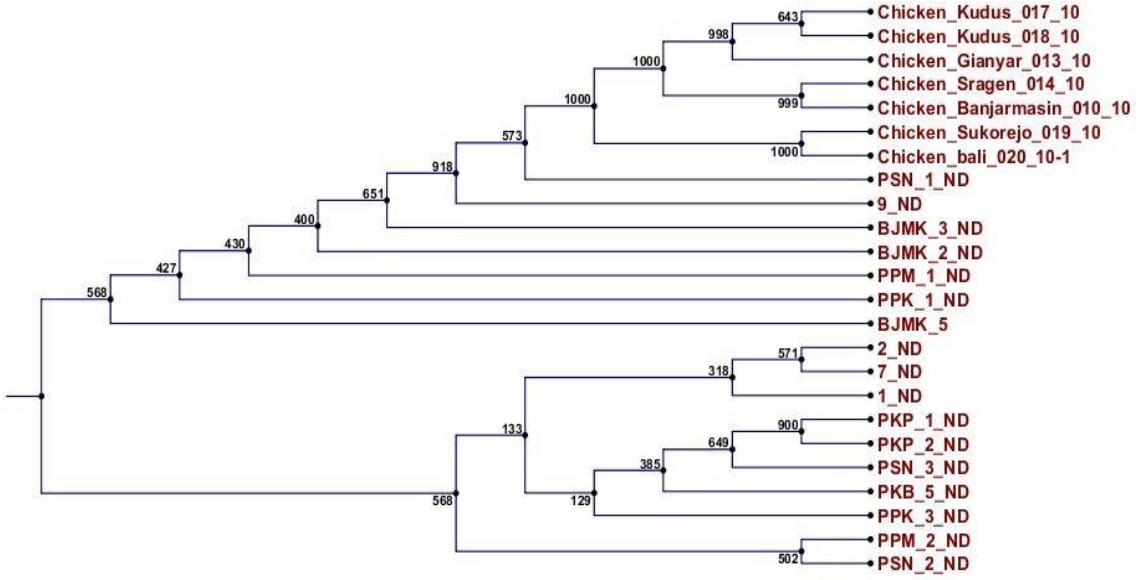
TGGAGGATGTTGGCAGCATTTTGGTTGGCTTGTATTATAGGCCGCAGCTGCTGTTATCTGTGCCGCTGTCGCAACCCCGAG
AGCTACACTGCCAATAACGGCACCTATAAAGCGTTTCTGTCTCCTTCCTCCGGACGTGGCCACCGACCCTTGGATCTTACG
GATGGAATCGCCAAGGGGAGTGAGCAAGGTAGTCAGTGTTCTGTTGTATGCCTCTAATGGGGCTTTTGCACACGCCYCTT
TATCCTTGGGCATATTCGGGAGCAACTTGACTATGATTGACCCTGTCTGAGATGAGGTGTATACATTGACTGCCTTATCTC
CTGTTMMTACAAKCCCTGCAGCTGCAAGAGGCTTGCCATCAAAA

Table 1. Homology Evalution in East Java compared to the last ND outbreak in Indonesia in 2010

ISOLATE	Banjarmasin/010/10	Sukorejo/019/10	Gianyar/013/10	Sragen/014/10	Kudus/017/10	Kudus/018/10	Bali/020/10
ktM 1 (1)	98%	-	98%	98%	98%	98%	-
tpM 3 (2)	99%	-	99%	99%	99%	99%	-
ktM 1 (3)	98%	-	98%	98%	98%	98%	-
tpM 3 (4)	99%	-	99%	99%	99%	99%	-
trM 1 (5)	98%	-	98%	98%	98%	98%	-
trM 1 (6)	98%	-	98%	98%	98%	98%	-
kdT 1 (7)	98%	-	98%	98%	98%	98%	-
KdB10 (9)	-	94%	-	-	-	-	94%
BJMK_2_ND	94%	-	94%	94%	94%	94%	-
BJMK_3_ND	95%	-	95%	95%	95%	95%	-
BJMK_5_ND	97%	-	97%	97%	97%	97%	-
PKB_5_ND	97%	-	97%	97%	97%	97%	-

PKP_1_ND	98%	-	98%	98%	98%	98%	-
PKP_2_ND	98%	-	98%	98%	98%	98%	-
PPK_1_ND	98%	-	98%	98%	98%	98%	-
PPK_3_ND	97%	-	97%	97%	97%	97%	-
PPM_1_ND	94%	-	94%	94%	94%	94%	-
PPM_2_ND	98%	-	98%	98%	98%	98%	-
PSN_1_ND	99%	-	99%	99%	99%	99%	-
PSN_2_ND	98%	-	98%	98%	98%	98%	-
PSN_3_ND	98%	-	98%	98%	98%	98%	-

Figure 7. Phylogeny Tree of East Java NVD isolates in the 2010 Outbreak in Indonesia



CONCLUSION

Based on the results above, it can be concluded that there are 20 isolates of NDV velogenicstarin in 20 districts of East Java Province and all codes of F gene (F protein). From the nucleotide bases and the phylogeny tree, the 20 isolates have close relation to Bojonegoro, Sukorejo, Gianyar (Bali Province), Sragen and Kudus isolates, but not to East Java isolates.

Conflict of Interest

The authors declare that there is no financial and personal relationships with other people or organizations that might inappropriately influence or bias their work.

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