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Research Reports

Isolation and Biological Characterization of Newcastle Disease Virus (NDV) Field Isolate Pigeon *(Columba livia)* from Live Bird Market, East Java in 2019

Isolasi dan Karakterisasi Biologis *Newcastle Disease Virus* (NDV) Isolat Lapang Burung Merpati *(Columba livia)* dari Pasar Unggas di Jawa Timur Tahun 2019

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ABSTRACT

Background: Avian Paramyxovirus (APMV) type-1 is the main cause of Newcastle Disease (ND) and taxonomically belongs to the family Paramyxoviridae, genus Avulavirus. Newcastle Disease (ND) is included in the A list by the OIE owing to its high transmission rate. **Purpose:** To determine the biological, characterize the Newcastle Disease Virus (NDV) field isolate of pigeons *(Columba livia)* using Mean Death Time (MDT), Intracerebral Pathogenicity Index (ICPI), and Intravenous Pathogenicity Index (IVPI). **Methods:** Twenty pigeon organ samples were obtained from bird markets in East Java and one was used as a positive control (LaSota). Organs were isolated from embryonated chicken eggs, identified by the HA test, and then confirmed by the HI test. Furthermore, positive samples were tested for MDT with a 10-1-10-18 dilution (0.1 ml and observed for eight days. The ICPI test used a fresh NDV suspension (0.05 ml and was observed for 8 days. The IVPI test used a dose of 0.1 ml and was observed for 10 days. **Results:** The MDT values of isolates MB1/NDV/19, MB2/NDV/19, MB3/NDV/19, and MG1/NDV/19 were 91.2 hours, 112.8 hours, 110.4 hours, and 124,8 hours. The ICPI values of isolate MB1/NDV/19 was 0.2375, MB2/NDV/19 was 0.375, MB3/NDV/19 was 0.5375, and MG1/NDV/19 was 0.3. The IVPI values of isolate MB1/NDV/19 was 0, MB2/NDV/19 was 0, MB3/NDV/19 was 0, and MG1/NDV/19 was 0. **Conclusion:** All four field samples were positive for NDV as a lentogenic strain based on the MDT, ICPI, and IVPI tests.

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ABSTRAK

Latar Belakang: *Avian Paramyxovirus* (APMV) type-1 merupakan penyebab utama penyakit Newcastle Disease (ND), dan secara taksonomi termasuk famili Paramyxoviridae, genus Avulavirus. Newcastle Disease (ND) masuk kedalam A-list oleh OIE karena tingkat penularan yang tinggi. **Tujuan:** Untuk mengetahui karakterisasi biologis virus ND isolat lapang burung merpati *(Columba livia)* melalui uji *Mean Death Time* (MDT), *Intracerebral Pathogenicity Index* (ICPI), dan *Intravenous Pathogenisicity Index* (IVPI). **Metode:** Sebanyak 20 sampel organ burung merpati diperoleh dari pasar burung di Jawa Timur dan satu kontrol positif (LaSota). Organ diisolasikan ke dalam telur Ayam Berembrio dan diidentifikasi melalui Uji HA, kemudian di konfirmasi dengan Uji HI. Selanjutnya, sampel positif diuji MDT dengan pengenceran 10-1-10-18 dosis 0,1 ml dan diamati selama 8 hari. Uji ICPI menggunakan suspensi virus ND segar dengan dosis 0,05 ml dan diamati selama 8 hari. Uji IVPI menggunakan dosis 0,1 ml dan diamati selama 10 hari. **Hasil:** Nilai MDT isolat MB1/NDV/19, MB2/NDV/19, MB3/NDV/19, dan MG1/NDV/19 berturut-turut adalah 91,2 jam, 112,8 jam, 110,4 jam, dan 124,8 jam. Nilai ICPI isolat MB1/NDV/19 sebesar 0,2375, MB2/NDV/19 sebesar 0,375, MB3/NDV/19 sebesar 0,5375, dan MG1/NDV/19 sebesar 0,3. Nilai IVPI isolat MB1/NDV/19 adalah 0, MB2/NDV/19 adalah 0, MB3/NDV/19 adalah 0, dan MG1/NDV/19 adalah 0. **Kesimpulan:** Disimpulkan bahwa ke empat sampel lapang positif virus ND dinyatakan sebagai strain lentogenik berdasarkan uji MDT, ICPI, dan IVPI.

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Kata kunci: Burung Merpati; Karakterisasi Biologi; Pasar Burung; Penyakit Newcastle; Strain Lentogenik

INTRODUCTION

Avian Paramyxovirus (APMV) type-1 is the main cause of Newcastle Disease (ND). Taxonomically, the ND virus belongs to the Paramyxoviridae family, Avulavirinae subfamily, and genus Avulavirus. Newcastle Disease (ND) has been identified as having 21 serotypes, such as APMV 1-21 and is known to infect more than 200 species of birds, including the Columbidae family (Columbiformis) (Amarasinghe *et al.,* 2019; OIE, 2021). The ND virus is divided into several strains based on the degree of virulence, ordered from high to low pathogenicity, namely Viscerotropic Velogenic, Neurotropic Velogenic, Mesogenic, Lentogenic or respiratory, and subclinical (OIE 2021). The structure of the ND virus is circular, pleomorphic, diameter 100-500 nm, enveloped, negative polarity, non-segmented genome, ss-RNA with six main proteins: NP, P, M, F, HN, L, and two non-structural proteins, namely V and W (Snoeck *et al.,* 2013; Ashraf and Shah, 2014; He *et al.,* 2018;).

The Office International des Epizooties (OIE, 2009) has classified the ND virus as an A-list disease because of its high infectivity, high mortality, and morbidity in poultry (OIE, 2009). Pigeons (Columbidae) are usually unvaccinated, live wild in nature, and act as reservoirs for ND virus antigens. In the Columbidae bird group, the ND virus is known as Pigeon Paramyxoviruses 1 (PPMV-1) (Wang *et al.,* 2021). PPMV-1 has a morbidity rate of up to 100% and a mortality rate of approximately 70%, with torticollis being the characteristic clinical feature (Guo *et al.,* 2014). The genus Columba is the main host species; however, several studies have shown that cross-infection occurs in chickens, leading to malignant traits (Brown and Bevins, 2017; Xiang *et al.,* 2019).

The pathogenicity of the ND virus in causing death in embryonated chicken eggs or Mean Death Time (MDT) is divided into velogenic strains (<60 h), mesogenic strains (60-90 hours), and lentogenic strains (>90 h) (Alazawy and Al Ajeeli, 2020; OIE, 2009). The ability of the ND virus to cause death of 1-Day-Old Chick (DOC) through the Intracerebral Pathogenicity Index (ICPI) test, with an assessment category that ICPI>1.5 was declared a velogenic strain, 0.7<ICPI<1.5 was declared a mesogenic strain, ICPI<0.7 was declared a lentogenic strain (Hossain *et al.,* 2017; OIE, 2009). The ability of the ND virus to cause death of 6-week-old chickens was assessed through the Intravenous Pathogenicity Index (IVPI) test, with the following assessment categories IVPI>1.45 was declared, velogenic strain; 0<IVPI<1.45 was declared, mesogenic strain; and IVPI=0, lentogenic strain (Awad *et al.,* 2015; OIE, 2009). Based on the above background, this study aimed to explain the biological characteristics of field isolates of pigeon (*Columba livia*) ND virus using pathogenicity tests, including MDT, ICPI, and IVPI.

MATERIAL and METHOD

Sample Collection

The samples were collected using a purposive sampling method. In this study, several organ samples of non-vaccinated pigeon (*Columba livia*) collected from bird markets in East Java were used. The samples collected in this study were obtained from necropsy pigeons (*Columba livia*). Several organs were collected, such as the brain, trachea, lungs, liver, proventriculus, and intestine

Sample Preparation

Organ samples were mashed into a 10% suspension (1 g organ + 9 ml PZ) and then stored in a microtube containing penicillin (2000 IU/ml) (Kimia Farma, INA) and streptomycin sulfate (200 µg/ml) (Kimia Farma, INA) as media. The samples were then stored at -80°C. Samples were isolated using the standard virus isolation method from Embryo Chicken Eggs (Miller *et al.,* 2015; OIE, 2009).

Hemagglutination Test

Hemagglutination test (HA) was performed according to the OIE protocol. Positive HA samples were then tested using the hemagglutination inhibition test (HI) to confirm the presence of the Newcastle Disease (ND) virus (OIE, 2021).

Mean Death Time

The Mean Death Time (MDT) was calculated to test the pathogenicity of ND virus in Embryonated Chicken Eggs. Inoculate 0.1 ml suspension on five Embryonated Chicken Eggs per dilution. Observations were made every 12 h for seven days (OIE, 2009; Roohani *et al.,* 2015). The suspension was titrated to obtain a 10-1-10-18 dilution

$$MDT = \frac{(\Sigma \text{ Eggs dead at } n \text{ 1 hours}) \times (n \text{ 1 hours}) + (\Sigma \text{ Eggs dead at } n \text{ 2 hours}) \times (n2 \text{ hours})..etc}{\Sigma \text{ Total Dead Eggs}}$$

Intracerebral Pathogenicity Index

The Intracerebral Pathogenicity Index (ICPI) was used to test the pathogenicity of the ND virus in 10 DOCs aged 1 day. A fresh suspension of ND virus with a titer of not less than 2^4 was injected intracerebral into 10 DOCs at as much as 0.05 ml. Observations were made for 8 days every 24 h for eight days (OIE, 2009; Roohani *et al.,* 2015). *(a = normal, b = sick, c = dead).*

$$ICPI = \frac{a + b + c}{\Sigma \text{ Total DOC x Number of Observation Days}}$$

Intravenous Pathogenicity Index

Intravenous Pathogenicity Index (IVPI) was performed to test the pathogenicity of the ND virus in 10 chickens aged 6 weeks. A fresh suspension of ND virus with a titer of not less than 2^4 was intravenously injected into 10 chickens aged 6 weeks at a dose of 0.1 ml. Observations were made every 24 h for 10 d (OIE, 2009). (a = normal, b = sick, c = paralysis, d = dead).

$$IVPI = \frac{a + b + c + d}{\Sigma \text{ Total Chickens x Number of Observation Days}}$$

RESULT

Isolation of ND Virus from Pigeons *(Columba livia)*

A total of 20 samples of pigeons (*Columba livia*) from bird markets in East Java (four samples from the Bratang Bird Market, Surabaya City; five samples from Candi District, Sidoarjo Regency; three samples from Sidoarjo District,

Sidoarjo Regency; three samples from Gresik Regency; four samples from Krian District, Sidoarjo Regency, and one sample from Pasuruan Regency), and five organs were taken for each sample. The organs examined in this study were the brain, respiratory organs (trachea-pulmo), hepar, and digestive organs (proventriculus and intestine). The organs were crushed and placed in 1.5 mL centrifuge tubes containing 1.0 mL of transport medium and labeled. The total number of organs used in this study comprised 100 organs from six regions.

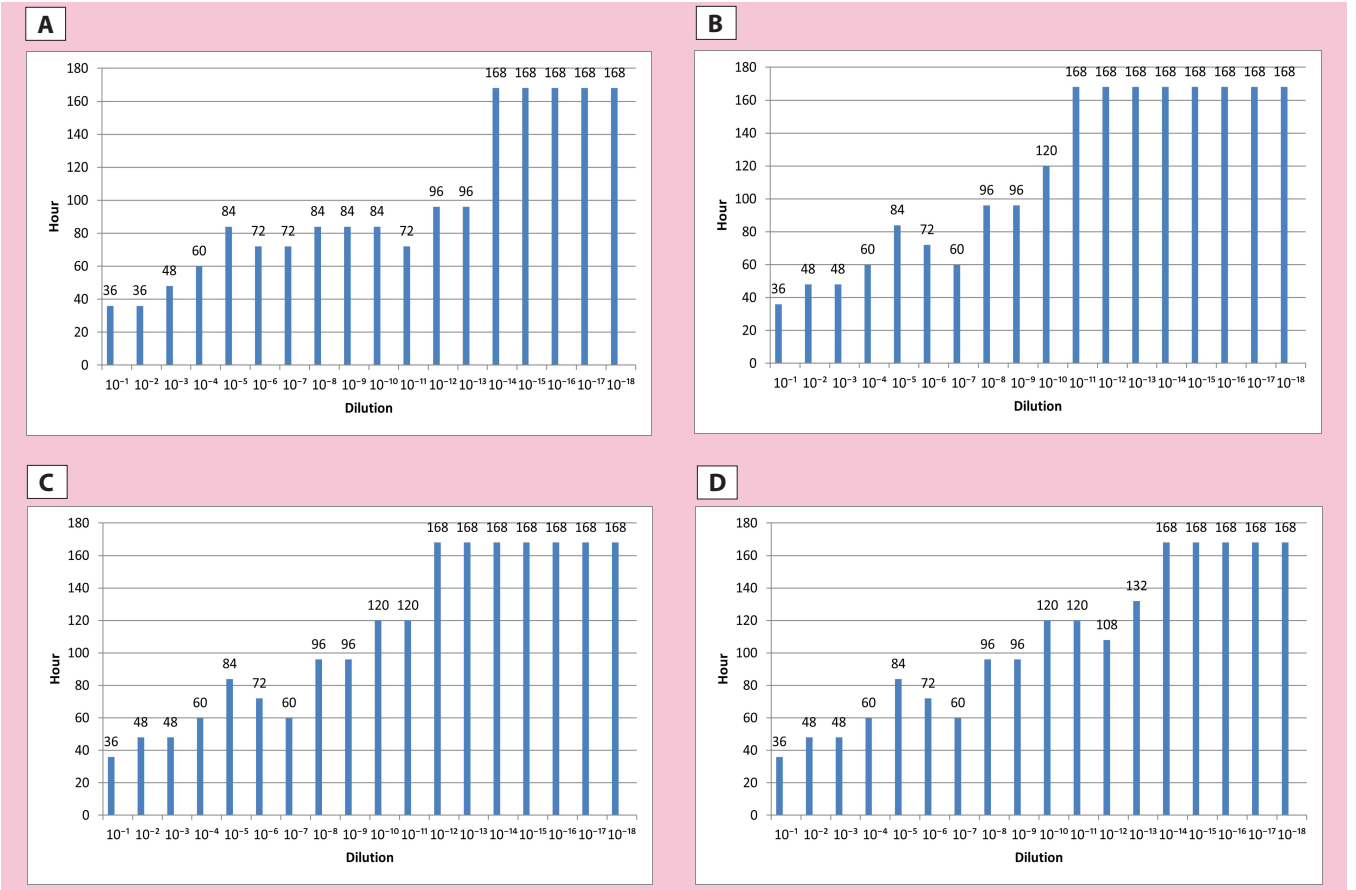
The organs were processed into a 10% suspension and then inoculated into 9 days old Embryonated Chicken Eggs for 120 h, with three Embryonated Chicken Eggs per organ. A total of 300 eggs were used. Egg candling once per day. If the eggs died during the incubation period (<120 h), the eggs were placed in the refrigerator at 4°C for 24 h, the allantoic fluid was collected from the eggs, and checked through the HA-HI test using the microtechnical method. Eggs that were still alive on the last day of the incubation period (120 h) were placed in a refrigerator at 4oC °C for 24 h, and the allantoic liquid was collected and examined using a microtechnical HA-HI test. The first step was the HA test, followed by the HI test using NDV antiserum. Based on the results of the HA-HI test, four out of 20 samples (11 organs) were positive for the ND virus, as described in Table 1.

Mean Death Time (MDT) Test

The Mean Death Time (MDT) was calculated for 90 Embryonated Chicken Eggs, with each dilution of five eggs. Before inoculating the eggs, the suspension was titrated until a dilution was obtained starting from 10-1-10-18. The dilution is based on the fact that the results of a 10-10 dilution still result in complete death (100%). A total of 0.1 ml (10-1 dilution) was inoculated into 5 eggs aged 9-11 days. The same procedure was performed for the 10-2 to 10-18 dilutions. Egg observations were carried out every 12 h for ± 7 days using an egg candler to check whether dead embryos were present (OIE, 2009). In this study, 100% above 10-10 dilutions. Dead eggs were stored in a refrigerator. At the end of the observation period, the remaining live eggs were placed in the refrigerator for further HA testing. The data collected from the Mean Death Time (MDT) observations of all positive isolates are shown in the following graph (Picture 1).

Intracerebral Pathogenicity Index (ICPI) Test

The Intracerebral Pathogenicity Index (ICPI) was calculated for 10 DOCs aged 1 day (OIE, 2009; Roohani *et al.*, 2015). A fresh suspension of Newcastle disease (ND) virus with a titer of not less than 2^4 was injected intracerebral as much as 0.05 ml. Observations were made for 8 days every 24 h for eight days, and the DOC conditions were observed. In this study,



Picture 1. Mean Death Time (MDT) graph. A. Isolate MB1/NDV/19. B. Isolate MB2/NDV/19. C. Isolate MB3/NDV/19. D. Isolate MG1/NDV/19.

Table 1.
The HA-HI test results were positive from pigeons (*Columba livia*) isolate.

Samples		Organs	HA Titre	HI Titre	Status
Bratang Bird Market, Surabaya City	MB1/ND/19	Brain	2^8	2^5	Positive
		Pulmo-Trachea	2^0	2^0	Negative
		Hepar	2^0	2^0	Negative
		Proventrikulus	2^0	2^0	Negative
		Intestine	2^8	2^5	Positive
	MB2/ND/19	Brain	2^{10}	2^6	Positive
		Pulmo-Trachea	2^0	2^0	Negative
		Hepar	2^0	2^0	Negative
		Proventrikulus	2^{10}	2^6	Positive
		Intestine	2^{10}	2^6	Positive
	MB3/ND/19	Brain	2^{10}	2^6	Positive
		Pulmo-Trachea	2^0	2^0	Negative
		Hepar	2^0	2^0	Negative
		Proventrikulus	2^{10}	2^6	Positive
		Intestine	2^{10}	2^6	Positive
	MBh1/ND/19	Brain	2^0	2^0	Negative
		Pulmo-Trachea	2^0	2^0	Negative
		Hepar	2^0	2^0	Negative
		Proventrikulus	2^0	2^0	Negative
		Intestine	2^0	2^0	Negative
Pasuruan	Mps1/NDV/19	Brain	2^0	2^0	Negative
		Pulmo-Trachea	2^0	2^0	Negative
		Hepar	2^0	2^0	Negative
		Proventrikulus	2^0	2^0	Negative
		Intestine	2^0	2^0	Negative

Samples		Organs	HA Titre	HI Titre	Status
Candi, Sidoarjo	MCn1/NDV/19	Brain	2^0	2^0	Negative
		Pulmo-Trachea	2^0	2^0	Negative
		Hepar	2^0	2^0	Negative
		Proventrikulus	2^0	2^0	Negative
		Intestine	2^0	2^0	Negative
	MCn2/NDV/19	Brain	2^0	2^0	Negative
		Pulmo-Trachea	2^0	2^0	Negative
		Hepar	2^0	2^0	Negative
		Proventrikulus	2^0	2^0	Negative
		Intestine	2^0	2^0	Negative
	MCn3/NDV/19	Brain	2^0	2^0	Negative
		Pulmo-Trachea	2^0	2^0	Negative
		Hepar	2^0	2^0	Negative
		Proventrikulus	2^0	2^0	Negative
		Intestine	2^0	2^0	Negative
	MCn4/NDV/19	Brain	2^0	2^0	Negative
		Pulmo-Trachea	2^0	2^0	Negative
		Hepar	2^0	2^0	Negative
		Proventrikulus	2^0	2^0	Negative
		Intestine	2^0	2^0	Negative
	MCn5/NDV/19	Brain	2^0	2^0	Negative
		Pulmo-Trachea	2^0	2^0	Negative
		Hepar	2^0	2^0	Negative
		Proventrikulus	2^0	2^0	Negative
		Intestine	2^0	2^0	Negative
Sidoarjo, Sidoarjo	MSk1/NDV/19	Brain	2^0	2^0	Negative
		Pulmo-Trachea	2^0	2^0	Negative
		Hepar	2^0	2^0	Negative
		Proventrikulus	2^0	2^0	Negative
		Intestine	2^0	2^0	Negative
	MSk2/NDV/19	Brain	2^0	2^0	Negative
		Pulmo-Trachea	2^0	2^0	Negative
		Hepar	2^0	2^0	Negative
		Proventrikulus	2^0	2^0	Negative
		Intestine	2^0	2^0	Negative
	MSk3/NDV/19	Brain	2^0	2^0	Negative
		Pulmo-Trachea	2^0	2^0	Negative
		Hepar	2^0	2^0	Negative
		Proventrikulus	2^0	2^0	Negative
		Intestine	2^0	2^0	Negative

the DOC isolates MB1/NDV/19 died on the 7th day, MB2/NDV/19 died on the 5th day, and MB3/NDV/19 died on the 2nd day, whereas isolates MG1/NDV/19 died on the 6th day. Data collected from ICPI observations of all positive isolates are shown in the following graph (Picture 2).

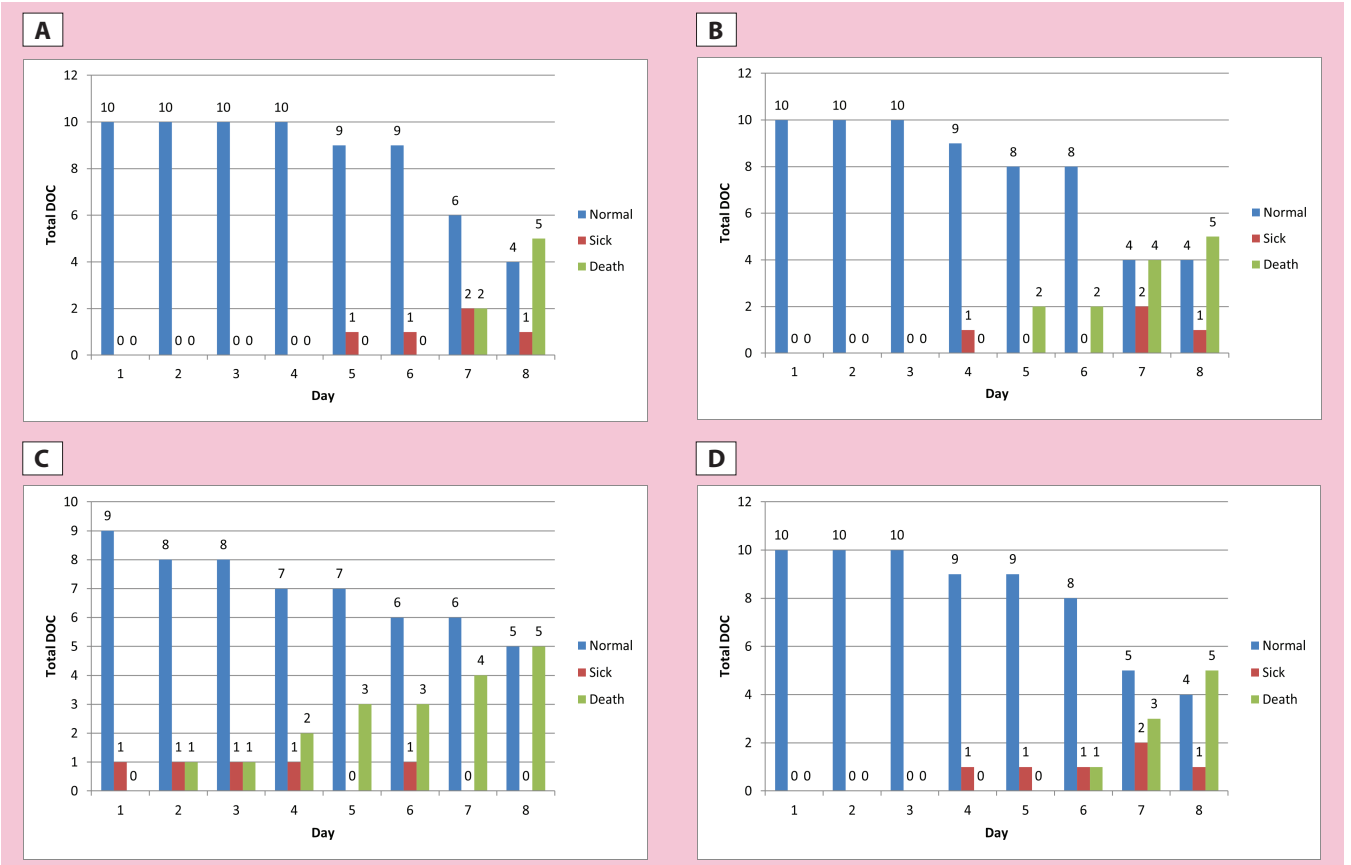
Samples		Organs	HA Titre	HI Titre	Status
Gresik	MG1/NDV/19	Brain	2^{10}	2^6	Positive
		Pulmo-Trachea	2^0	2^0	Negative
		Hepar	2^0	2^0	Negative
		Proventrikulus	2^{10}	2^6	Positive
		Intestine	2^9	2^6	Positive
	MG2/NDV/19	Brain	2^0	2^0	Negative
		Pulmo-Trachea	2^0	2^0	Negative
		Hepar	2^0	2^0	Negative
		Proventrikulus	2^0	2^0	Negative
		Intestine	2^0	2^0	Negative
	MG3/NDV/19	Brain	2^0	2^0	Negative
		Pulmo-Trachea	2^0	2^0	Negative
		Hepar	2^0	2^0	Negative
		Proventrikulus	2^0	2^0	Negative
		Intestine	2^0	2^0	Negative
Krian, Sidoarjo	Mkri1/NDV/19	Brain	2^0	2^0	Negative
		Pulmo-Trachea	2^0	2^0	Negative
		Hepar	2^0	2^0	Negative
		Proventrikulus	2^0	2^0	Negative
		Intestine	2^0	2^0	Negative
	Mkri2/NDV/19	Brain	2^0	2^0	Negative
		Pulmo-Trachea	2^0	2^0	Negative
		Hepar	2^0	2^0	Negative
		Proventrikulus	2^0	2^0	Negative
		Intestine	2^0	2^0	Negative
	Mkri3/NDV/19	Brain	2^0	2^0	Negative
		Pulmo-Trachea	2^0	2^0	Negative
		Hepar	2^0	2^0	Negative
		Proventrikulus	2^0	2^0	Negative
		Intestine	2^0	2^0	Negative
	Mkri4/NDV/19	Brain	2^0	2^0	Negative
		Pulmo-Trachea	2^0	2^0	Negative
		Hepar	2^0	2^0	Negative
		Proventrikulus	2^0	2^0	Negative
		Intestine	2^0	2^0	Negative

Intravenous Pathogenicity Index (IVPI) Test

Intravenous Pathogenicity Index (IVPI) was performed on 10 chickens aged 6 weeks. A fresh suspension of Newcastle disease virus (ND) with a titer not less than 2^4 mL was injected intravenously as much as 0.1 ml (OIE, 2009). Observations were made every 24 h for 10 days, and the condition of the chickens was monitored. In this study, chicken isolates MB1/NDV/19, MB2/NDV/19,MB3/NDV/19, and MG1/NDV/19 did not cause any illness or death. Data collected from the IVPI observations of all positive isolates are shown in the following graph (Picture 3)

DISCUSSION

Definitive assessment of Newcastle Disease (ND) virus virulence from Bratang isolates (MB1/NDV/19, MB2/NDV/19, and MB3/NDV/19) and Gresik isolate (MG1/NDV/19) showed different time variations (MDT Graph A-D). Ninety Embryonated Chicken Eggs per isolate died between 84 and 156 h. The Mean Death Time (MDT) isolate MB1/NDV/19 had an average death of 91.2 hours. The MB2/NDV/19 isolate Mean Death Time (MDT) has an average death time of 112.8 hours. The MB3/NDV/19 isolate Mean Death Time (MDT) has an average death time of 110.4 hours, and the Mean Death Time (MDT) isolate MG1/NDV/19 has an average death rate of 124.8 hours. From the results of the average mortality of



Picture 2. Mean Death Time (MDT) graph. A. Isolate MB1/NDV/19. B. Isolate MB2/NDV/19. C. Isolate MB3/NDV/19. D. Isolate MG1/NDV/19.

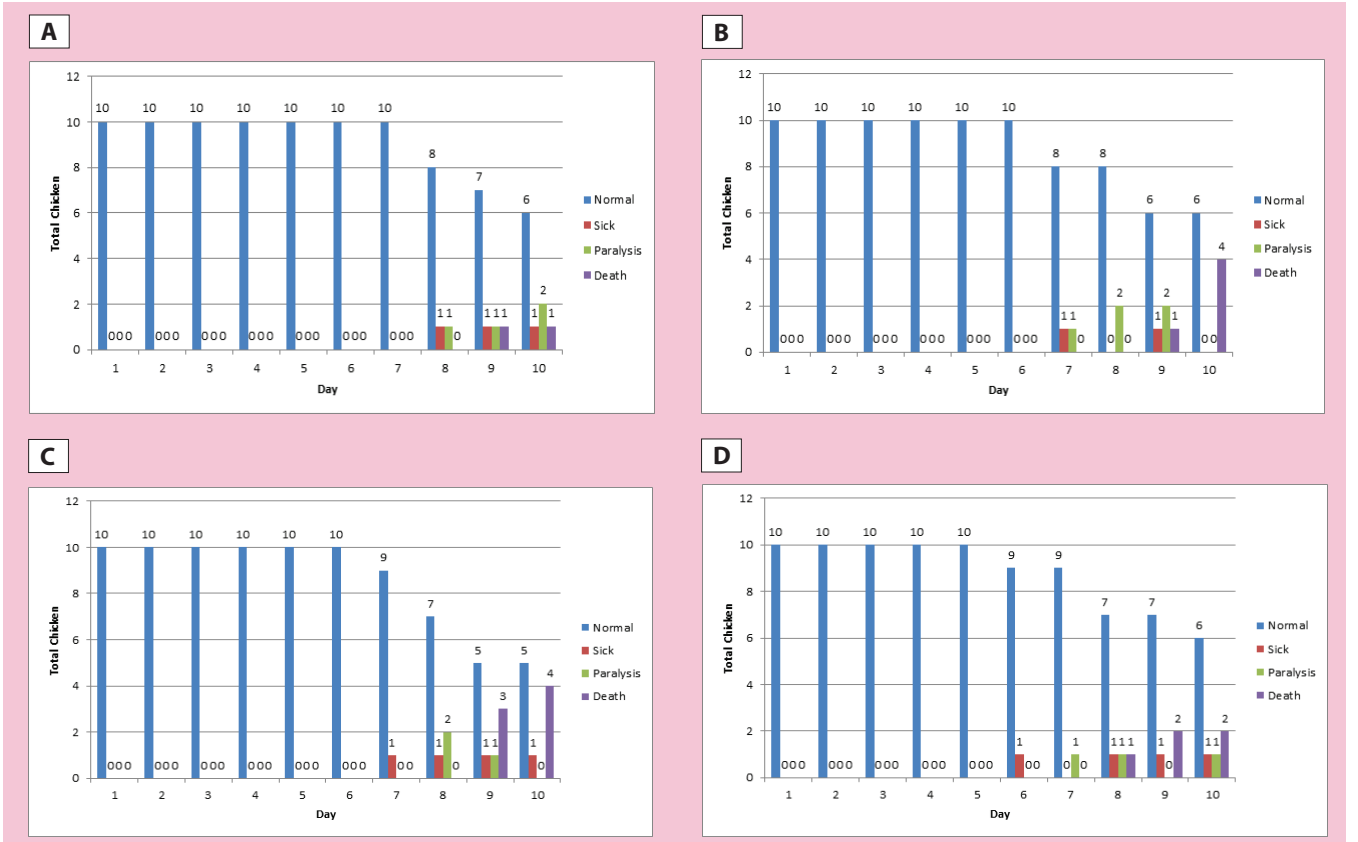
the four isolates above, it can be concluded that the Bratang isolates (MB1/NDV/19, MB2/NDV/19, and MB3/NDV/19) and the Gresik isolate (MG1/NDV/19) fell into the category lentogenic pathotype. The pathogenicity of the ND virus as a lentogenic pathotype is based on its ability to cause death in Embryonated Chicken Eggs (MDT) for more than 90 h (Almubarak, 2019).

HA testing was carried out on the four isolates to check for the presence of hemagglutinin produced by the Newcastle disease (ND) virus, which was inoculated in allantoic fluid. The HA test results for the four positive isolates were shown by the presence of complete hemagglutination (diffuse) and without erythrocyte precipitate. The Mean Death Time (MDT) was calculated based on the number of embryonated chicken eggs that died at the highest dilution tested.

Newcastle Disease (ND) virus pathogenicity test through the Intracerebral Pathogenicity Index (ICPI), using a fresh suspension with a titer of not less than 2^4 intracerebral doses of 0.05 ml. The results of this study show varying values (ICPI Graph A-D). The results of the observation of the Intracerebral Pathogenicity Index (ICPI) of MB1/NDV/19 isolate was 0.2375. The Intracerebral Pathogenicity Index (ICPI) value of the MB2/NDV/19 isolate was 0.375. The Intracerebral Pathogenicity Index (ICPI) value of the MB3/NDV/19 isolate was 0.5375. The Intracerebral Pathogenicity Index (ICPI) of isolate MG1/NDV/19 was 0.3. From these observations, it can be concluded that the Bratang isolates (MB1/NDV/19, MB2/NDV/19, and MB3/NDV/19) and Gresik isolates (MG1/NDV/19) belong to the lentogenic pathotype. The Intracerebral Pathogenicity Index (ICPI) test category of ≤0.7 was declared a lentogenic strain (Almubarak, 2019).

Clinical symptoms are associated with the young age of DOC, when the immune system is not working properly (Schijns *et al.,* 2013). The most visible clinical symptoms during the incubation period on day 1 for DOC isolate MB3/NDV/19 were airsacculitis and facial swelling, followed by death on day 2. Clinical symptoms of airsacculitis for DOC isolates MB2/NDV/19 and MG1/NDV/19 were observed on the 4th day and followed by death the next day. Clinical symptoms of airsacculitis caused by DOC isolate MB1/NDV/19 were observed on day 5, followed by death on day 7.

The Intravenous Pathogenicity Index (IVPI) test uses a fresh suspension titer of not less than 2^4 intravenously in the brachial vein at a dose of 0.1 ml. The results of this study show the same value (IVPI Graph A-D). The results obtained from the observation of the Intravenous Pathogenicity Index (IVPI) of isolate MB1/NDV/19 was 0. The Intravenous Pathogenicity Index (IVPI) value of the isolate MB2/NDV/19 was 0. The Intravenous Pathogenicity Index (IVPI) value of the isolate MB3/NDV/19 was 0. The Intravenous Pathogenic-



Picture 3. Intravenous Pathogenicity Index (IVPI) graph. A. Isolate MB1/NDV/19. B. Isolate MB2/NDV/19. C. Isolate MB3/NDV/19. D. Isolate MG1/ND-

ity Index (IVPI) of isolate MG1/NDV/19 was 0. From these results, it was concluded that the Bratang isolates (MB1/NDV/19, MB2/NDV/19, and MB3/ NDV/19) and the Gresik isolate (MG1/NDV/19) belonged to the lentogenic pathotype. The IVPI test rating category that causes death in 6-week-old chickens was equal to 0 (IVPI=0) and was declared lentogenic (Awad *et al.*, 2015; OIE, 2009).

The immune system of 6-week-old chickens generally operates well. Therefore, clinical symptoms did not appear after the ND virus injection. The bursa of Fabrisius develops rapidly in young chicks and reaches its maximum size at 4-12 weeks of age. The largest mass in the bursa of Fabrisius is due to the large population of B lymphocyte cells that produce IgM (lymphoblasts), which undergo a maturation process. Young birds aged 2-8 weeks have a very active bursa of Fabrisius (Palya *et al.*, 2014).

CONCLUSION

Based on the research results, it can be concluded that the characteristics of the isolates MB1/NDV/19, MB2/NDV/19, MB3/NDV/19, and MG1/NDV/19 were based on the MDT, ICPI, and IVPI tests were declared Lentogenic strains.

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CONFLICT of INTEREST

The author declares no conflict of interest.

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ETHICAL APPROVAL

The ethical test was conducted at Airlangga University with ethical number No. KE105.07.2018.

AUTHORS' CONTRIBUTIONS

Acquisition of data: VN, FAR. Analysis and interpretation of data: VN, FAR. Drafting the manuscript: VN, FAR, S, RRE, JR. Revision manuscript: VVN, FAR, S, RRE, JR.

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