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Newcastle disease virus: the past and current situation in Indonesia

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ABSTRACT

The Newcastle disease virus (NDV) outbreak was first reported in Java Island, Indonesia, in 1926, which was then reported further in Newcastle-upon-Tyne, England. Nevertheless, the NDV is still endemic in Indonesia, with outbreaks occurring in free-range and commercial chicken farms. The dynamic evolution of the NDV has led to the further development of vaccines and diagnostic tools for more effective control of this virus. This paper discusses the history of the NDV occurrence, vaccines, the development of diagnostic tools, and the epidemiological condition of the NDV in Indonesia. Indonesia, which has the largest poultry population in the world after China, has challenges in preventing and controlling this virus that causes economic losses to the farmers and has an impact on the welfare of the poultry farming community in Indonesia.

Keywords: Vaccine; paramyxoviridae; birds; epidemiology; farming

INTRODUCTION

Newcastle disease (ND) is a highly contagious and economically important avian viral disease that was categorized as a list A disease of poultry by the Office International des Epizooties (OIE) [1]. Since its first emergence in Java Island, Indonesia [2] and Newcastle-upon-Tyne, England in 1926 [3,4], outbreaks of the disease have continued to spread globally, leading to substantial economic losses to the global poultry industry [5-7].

ND is a disease caused by the Newcastle disease virus (NDV), an avian orthoavulavirus 1 (AOAV-1) that belongs to the Family *Paramyxoviridae*, Subfamily *Paramyxovirinae*, Genus *Avulavirus* [1]. An outbreak of the NDV was first reported in Java Island, Indonesia, in 1926, which was then encountered in Newcastle-upon-Tyne, England [2,3]. Thus far, the NDV is still endemic in Indonesia, with outbreaks occurring in free-range and commercial chicken farms [4,5]. The NDV is an enveloped virus with a single-stranded, negative-strand RNA genome with a wide host range [6]. An NDV infection is initiated by receptor recognition and binding to the host cell surface. It is then followed by fusion, the interaction of two surface glycoproteins, called fusion protein (F) and hemagglutinin-neuraminidase (HN) [7]. This virus consists of two classes based on the complete nucleotide sequence of the F gene,

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Conflict of Interest

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namely class I and class II [8]. The class I viruses belong to a single genotype, whereas the more genetically diverse class II viruses are divided into 20 (I-XXI) genotypes [9].

NDV strains can be classified into five pathotypes based on their severity in chickens: viscerotropic and neurotropic velogenic (highest mortality rate, intracerebral pathogenicity index [ICPI] > 1.5), mesogenic (low mortality but with moderate clinical symptoms in the respiratory system, ICPI 1.5–0.7), lentogenic (mild respiratory infection without death, live-vaccine strains, ICPI < 0.7), and asymptomatic (no clinical symptoms or subclinical enteric infection) [10]. The NDV is highly contagious in poultry, with high morbidity and mortality, causing significant economic losses that impact food security in developing countries, including Indonesia [11].

Although the vaccination control program against the NDV in Indonesia has been carried out, outbreaks of ND still occur periodically in poultry farms. This paper reviews the history of the circulation of the NDV in Indonesia, the development of vaccines, and the diagnostic tools that follow so it can be used as a scientific basis for policymaking.

OVERVIEW OF THE NDV

Indonesia has a large poultry population, particularly for broilers, laying hens, and native chickens. As a country with the fifth largest human population in the world, with a Muslim majority, the poultry industry plays a vital role in the Indonesian economy. The poultry population in Indonesia has fluctuated from year to year (2000–2021) but tends to increase (**Fig. 1**). This poultry population is affected by various conditions, including

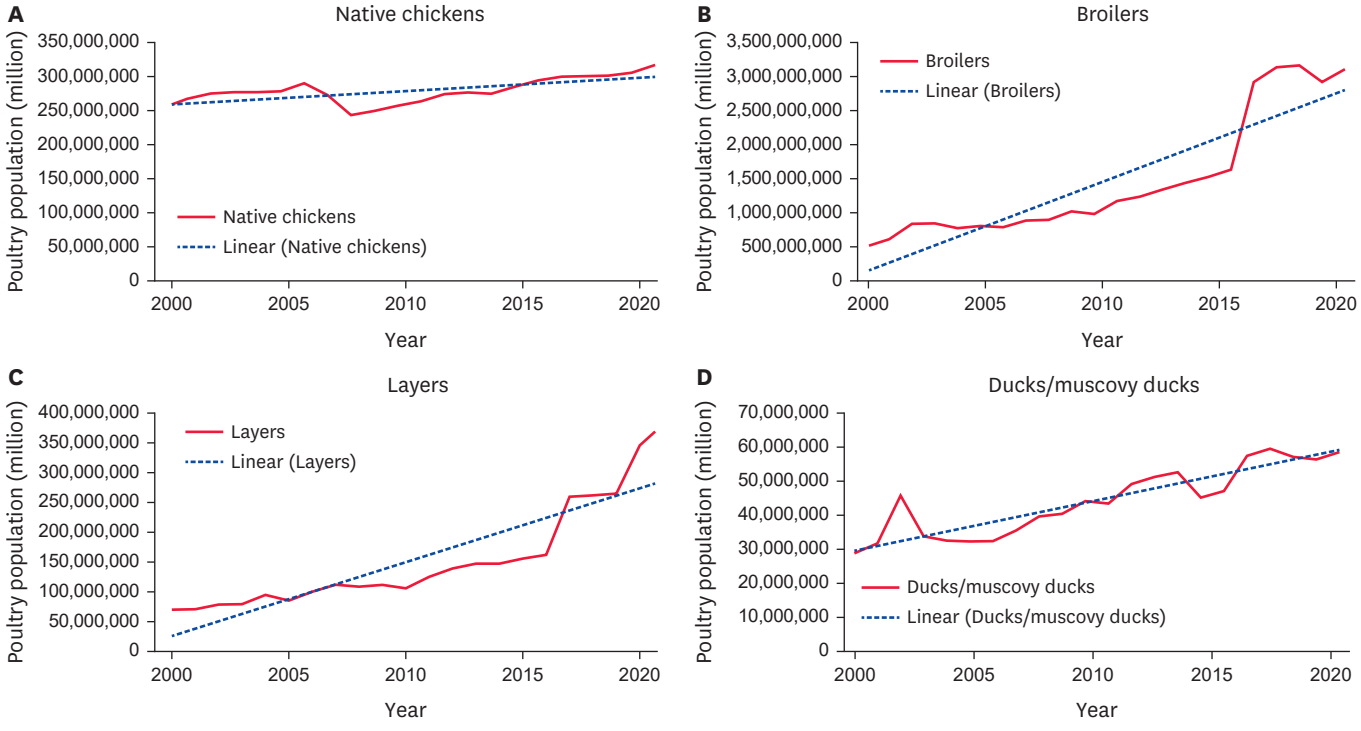

Fig. 1. Overview of the poultry population in Indonesia in 2000–2021.

infectious diseases in poultry. The NDV is also known as exotic Newcastle disease (END), pseudo-fowl pest, pseudo-vogelpest, atypische Geflügelpest, pseudo-poultry plague, avian pest, avian distemper, Ranikhet disease, Tetelo disease, Korean fowl plague, and avian pneumoencephalitis. ND is a viral disease of poultry that is endemic in Asia, Africa, the Middle East, and Central and South America, including Indonesia [12]. The viruses in wild birds and non-commercial poultry are often referred to as avian paramyxoviruses 1 (APMV-1) to distinguish them from viruses that cause deadly infections in poultry, commonly called virulent NDV. Subsequently, The International Committee on Taxonomy of Viruses (ICTV) officially changed the name NDV or APMV-1 to avian avulavirus 1 in 2016. It was referred to as AOAV-1 in 2018 [13]. This review still uses the term NDV to describe the AOAV-1 virus.

NDV VIRION

NDV virions are pleomorphic, some spherical with diameters between 100 and 500 nm or filamentous with diameters around 100 nm and vary in length [14]. It is an enveloped virus composed of a lipid bilayer with a single-stranded, negative-strand RNA genome that encodes six structural proteins: large polymerase protein (L), HN, F, matrix protein (M), phosphoproteins (P), and nucleoproteins (NP) [6,12]. The size of the NDV genome varies: 15,186 (class II genotypes I–IV, early isolates), 15,192 (class II genotypes V–VIII, latest isolates), or 15,198 nucleotides (class I). The length of its surface glycoprotein in spike form, which consists of F and HN proteins, is 8–17 nm [12,15]. In addition to the P gene that can be transcribed into three different mRNAs encoding one structural protein (P) and two nonstructural proteins (V and W), all other viral genes are monocistronic, encoding one structural protein. The 3' and 5' ends of the viral genome are regions that accommodate the regulatory signals for viral transcription and replication [15]. Membrane proteins (F and HN proteins) play a role in assembling and budding enveloped RNA viruses and are determinants of host range and tissue tropism. The HN protein plays a role in receptor recognition and viral neuraminidase activity. The HN protein is involved in recognizing receptors that have sialic acid on the cell surface and increase the activity of the F protein fusion, allowing the virus to penetrate the cell surface and act as a NA by removing sialic acid from progeny virus particles to prevent self-agglutination in progeny viruses [16]. The F protein is also a major determinant of pathogenicity and inducing protective immunity against NDV infection [17,18].

Of all NDV structural proteins, the M protein is the most abundant in the virion. It forms the outermost protein layer around the nucleocapsid, bridging the nucleocapsid and the viral envelope [19]. The viral matrix protein, which consists of 364 amino acids and has a molecular weight (MW) of approximately 40 kDa, is responsible for viral assembly, disassembly, budding, and interactions with lipid membranes. pH can influence the protein matrix layer to facilitate viral disassembly by the endocytic route [20]. NP proteins are also abundant in viral particles involved in viral transcription and replication [21]. The NP protein, along with other internal proteins, namely P protein and L protein, was reported to participate in NDV virulence [12]. During transcription of the P gene, two additional nonstructural proteins, V and W, are produced by mRNA editing. The P protein has many roles, is very important in synthesizing viral RNA, and forms a complex with NP and L proteins that encapsulate the viral RNA. The P protein interacts with the NP protein to ensure that the NP protein is in a soluble state, resulting in the production of virus-specific RNA. The P protein binds to the L protein, which helps increase the interaction between the L protein template and viral RNA [22]. A recent publication also showed that the P protein plays a role in the thermostability of NDVs [22].

The V protein of the NDV is a multifunctional protein associated with its role in viral replication, viral pathogenesis, virulence, and an interferon alpha antagonist. This protein is closely related to host range restriction, which can efficiently overcome the host's innate immune system [23]. The V protein plays a vital role in viral replication. Furthermore, protein V can prevent apoptosis in a species-manner *in vitro* [24]. On the other hand, the W protein is relatively diverse among NDV strains and is considered to play a role in its pathogenicity and virulence [25].

NDV GENOTYPE, SUB-GENOTYPE, AND PATHOTYPE

The wide circulation of the NDV in the poultry population causes significant genetic diversity of the virus and the constant emergence of its variants. Given the clinical and economic relevance of NDVs to the poultry industry and the widespread use of live vaccines worldwide, DNA sequencing, and phylogenetic analysis are the methods of choice for characterizing NDV strains [26].

NDVs are divided into class I and class II based on the nucleotide sequence of the F gene [8]. The class I virus mainly circulates in wild birds that do not receive ND vaccination. This can explain why the genetic diversity of class 1 NDVs is relatively lower than that of class II. The low virulence of class I NDV in poultry and wild birds and the low incidence in poultry have limited the number of studies on virus sequencing and characterization. Hence, the spatial distribution is relatively limited in most available sequences. On the other hand, class II NDVs are more diverse, consisting of both non-virulent and virulent strains, with at least 20 different genotypes (I–XXI, genotype XV that only has recombinant sequences were excluded from the final analysis) [26]. **Table 1** lists the genotype and sub-genotype of the NDV.

Before molecular testing and DNA sequencing were available, the pathotypes were grouped based on the results of pathogenicity tests, as discussed earlier. A virulent strain is defined by the World Organization for Animal Health (formerly the OIE) as a virus with an ICPI of 0.7 or higher (maximum 2.0) or a fusion cleavage site with multiple basic amino acids and phenylalanine at the location of 117 [10]. The intravenous pathogenicity index (IVPI) in six-week-old chickens has also been used to differentiate velogenic NDVs from mesogenic and lentogenic NDVs, but the IVPI is rarely used because mesogenic viruses, such as velogenic viruses, are defined as virulent NDVs by the OIE [10,12].

CLINICAL SYMPTOMS OF ND DISEASE

In 2018, the NDV was reported to have had a global impact on 109 of the 200 OIE member countries in the last five years [1,27]. Infections by this virus have been documented in at least 250 avian and bird species. The clinical symptoms of NDV can vary from the absence of clinical symptoms to clinical neurological signs, paralysis, and acute death. This depends on the infection, strain, infection dose, exposure route, host, immunological status, and environmental conditions (**Fig. 2**) [6]. The clinical symptoms from one bird species to another may differ. For example, in certain bird breeds, clinical disease in unvaccinated commercial turkeys may not be as severe as in specific-pathogen-free (SPF) turkeys [12].

Under laboratory conditions, chickens infected with the virulent NDV (vNDV) at low doses (10^2 EID_{50} per 0.1 mL) did not show clinical symptoms, and no death was found.

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Table 1. Class II NDV genotypes and sub-genotypes

Genotype	Sub-genotype
I	I.1.1
	I.1.2.1
	I.1.2.2
	I.2
II	-
III	-
IV	-
V	V.1
	V.2
VI	VI.1.1
	VI.1.2.1.2
	VI.1.2.2.1
	VI.1.2.2.2
	VI.2.1.1.1
	VI.2.1.1.2.1
	VI.2.1.1.2.2
VII	VII.1.1
	VII.1.2
	VII.2
VIII	-
IX	-
X	-
XI	-
XII	XII.1
	XII.2
XIII	XIII.1.1
	XIII.1.2
	XIII.2.1
	XIII.2.2
XIV	XIV.1
	XIV.2
XVI	-
XVII	-
XVIII	XVIII.1
	XVIII.2
XIX	-
XX	-
XXI	XXI.1.1
	XXI.1.2
	XXI.2

Class II NDV genotypes and sub-genotypes based on Dimitrov et al. [26].
NDV, Newcastle disease virus.

Under medium and high infectious doses (10^4 EID_{50} per 0.1 mL and 10^6 EID_{50} per 0.1 mL, respectively), however, the vNDV virus resulted in various clinical symptoms. The clinical symptoms observed in this group include lethargy, conjunctivitis, necrotic, and necrohemorrhagic in the legs and combs, neurological symptoms in the form of ataxia, tremors, and torticollis, difficulty breathing, and death [28].

The NDV can replicate in almost every organ and mainly affects the digestive, respiratory, and nervous systems, causing complex pathologies in these organs and decreasing growth and egg production. In female birds, the NDV replicates in the ovaries and oviducts, causing severe inflammation and apoptosis, resulting in decreased egg production and fertility. In male birds, however, NDV replicates in testicular tissue, increases the expression of pattern recognition receptors (PRR), enhances innate immune responses, induces histologic lesions, and inhibits steroidogenesis and spermatogenesis [29]. In quail (*Coturnix coturnix japonica*),

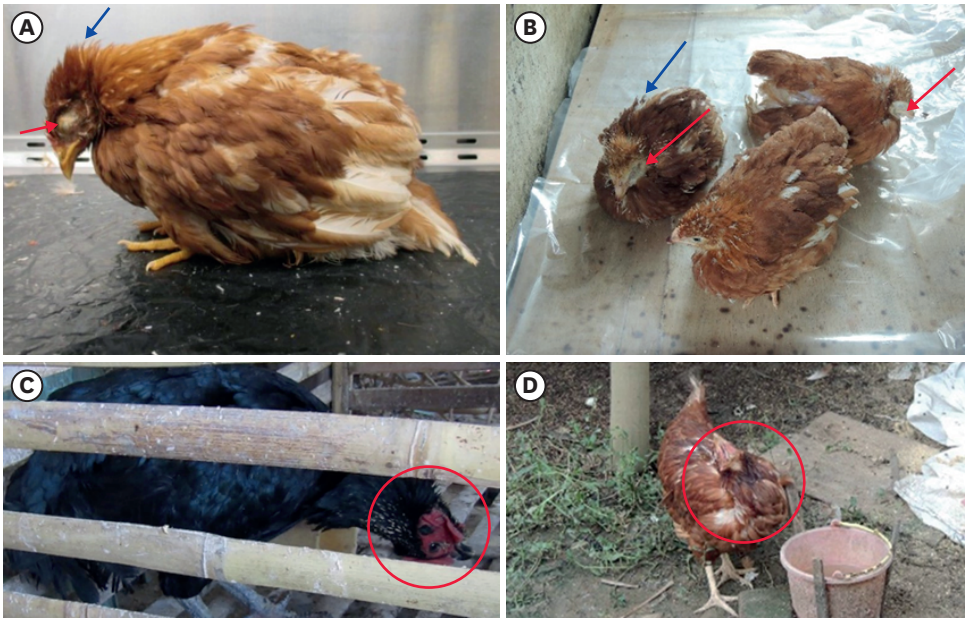

Fig. 2. Clinical symptoms such as lethargy, ruffled fur (blue arrow), swelling of the eyelid and tissues of the head (red arrow) (A, B) and torticollis (red circle) (C, D) were observed in chickens with NDV-confirmed infection. NDV, Newcastle disease virus.

the clinical signs of infection by the vNDV virus include depression, matted feathers, tracheal rales, leg paralysis, and torticollis observed seven days after infection [30]. Unvaccinated pheasants are very susceptible to the clinical symptoms found in chickens. The clinical signs reported in pheasants infected with the vNDV virus strain include neurological clinical signs, incoordination, depression, loss of appetite, watery white/green diarrhea, decreased egg quality and quantity, and head shaking. Mortality in pheasants ranges from 3% to 100% [31].

Ostriches generally show the clinical signs of infection in the nervous system, and death occurs only in young birds. Chicken isolates infecting pigeons were reported to cause neurological signs, such as tremors [12]. In pigeons, ND is caused by a pigeon-specific variant NDV known as pigeon paramyxovirus-1 (PPMV-1) as a genotype VI. Outbreaks in pigeons were first reported in the Middle East during the 1970s, spread to Europe during the 1980s, and are currently endemic worldwide. Generally, the most sensitive host will show more severe clinical signs than the less sensitive one. The order of the most sensitive hosts to the less sensitive were chickens, turkeys, pigeons, and ducks. Geese were generally grouped with ducks regarding sensitivity [12].

IDENTIFICATION OF NDV IN INDONESIA

Since being reported in 1926, NDV has caused four panzootic events worldwide, including in Asia, the Middle East, Africa, and Europe [32]. **Fig. 3** summarizes the history of NDV research in Indonesia. Despite the vaccination control program, the NDV is still a significant threat to the poultry industry worldwide, including Indonesia.

An outbreak of the disease occurred in Jakarta in March 1926 that caused considerable economic losses. The epidemic then spread to several cities east of Jakarta, including Bogor,

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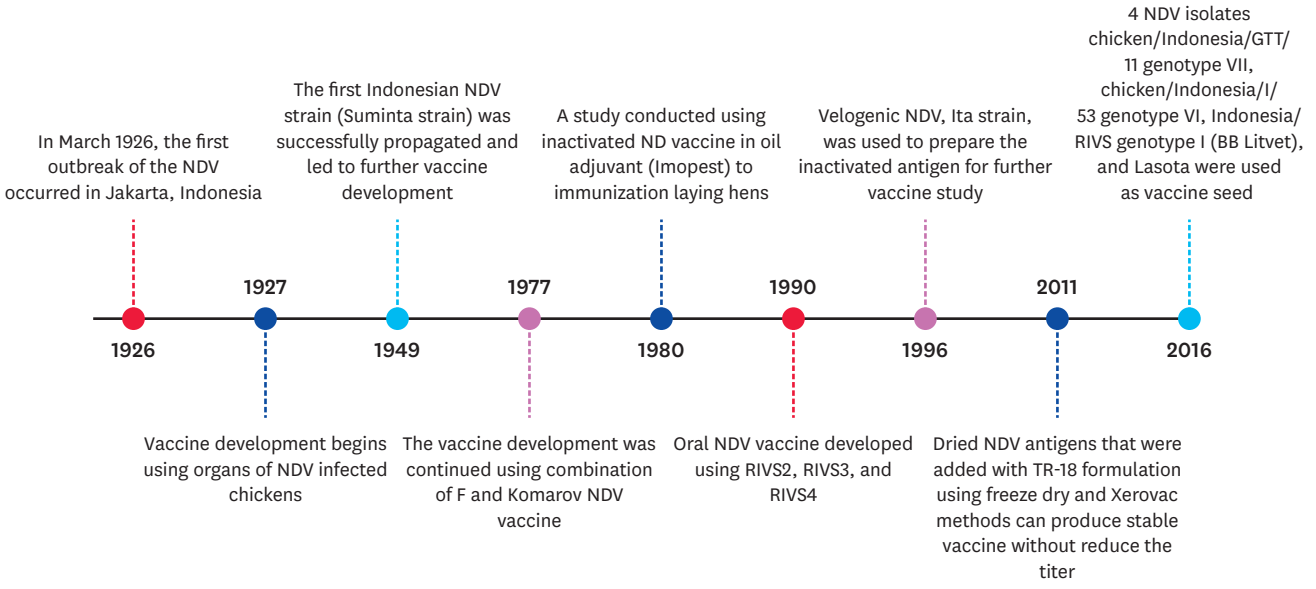

Fig. 3. Development of NDV research during 1926–1990 period in Indonesia.
ND, Newcastle disease; NDV, Newcastle disease virus.

Cirebon, Semarang, and Surabaya, infecting many animals, such as chickens, turkeys, and pheasants [33]. The post-mortem symptoms and lesions were similar to other fowl pests (e.g., avian influenza). On the other hand, there are differences between a more extended incubation period of 3–12 days, negative results from inoculation with blood and organs, and lack of immunity to fowl pests. This disease appears to be a new disease, later called "pseudo-fowl pest," which has clinical symptoms of paralysis, lethargy, facial edema, profuse diarrhea, and bleeding in the proventriculus in infected birds. It causes acute fever, which is highly infectious, with a mortality rate ranging from 90% to 100%.

Genotypes II, III, and IV of class II NDV contributed to the first panzootic from the 1920s to the 1960s. Genotypes V and VI were considered responsible for the occurrence of the second and third panzootic. The origin of the second panzootic was reported in the late 1960s and has been globally distributed over the past four years in Europe. The VIb subgenotype virus originated in the Middle East and contributed to the third panzootic event in pigeons during the 1980s. Viruses of genotype VII are responsible for the fourth panzootic occurrence, which continues today, and have spread from Asia, Africa, and Europe and have even been isolated in South America. The fourth panzootic started around 1985 in Southeast Asia and spread to most countries in Africa and Venezuela, South America. Genotypes VII and VIII have been responsible for the incidence of the outbreaks in Asia, including Pakistan, and in Europe since 1984. Genotypes V, VI, VII, VIII, and XI emerged after the 1960s and are considered 'late' genotypes, consisting only of the vNDV virus strain. The viruses of genotype VII are most associated with ND outbreaks occurring in the Middle East and Asia [34].

LOCAL VACCINE DEVELOPING

First vaccine development

Vaccine development commenced in 1927 with an organ vaccine using brain material, which attenuated/weakened the virus with lime, carbo glycerin, saline, and chloroform. On the

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other hand, the study did not achieve satisfactory results [33]. Various research methods have been implemented to answer problems related to vaccination-generated immunity. Some of these methods include: 1) the Mieszner–Baars-based process (dry chalk vaccine) and the Umeno-doi method (carboglyserin-saline vaccine), in which both methods are used in developing rabies immunization in dogs; 2) the Kelser process (chloroform vaccine) developed in the Philippines for rinderpest immunization; 3) the mitigation of pseudo fowl pest virus through injection in pigeons [35].

Various immunization methods have been reported: 1) Ether-virus vaccination based on Bailly's rabies vaccination using an artificially infected virulent virus from chicken brains; 2) vaccination using a very lethal dose of virus injected into quail feathers for slow absorption of the virus through integumentary contact; 3) bile vaccination based on KOCH's rinderpest vaccination using chicken bile taken from a cadaver; 4) immunization based on a bacteriophage scheme, in which the vaccine containing chicken fecal filtrate recovered from pseudo fowl pest infection, was reported with unsatisfactory results. On the other hand, immunization with quail feathers could protect against viral infections by 100 minimum lethal doses (MLDs) but could not cope with higher viral doses and natural infections [36].

In 1933, the use of vaccines prepared from the brain, spleen, liver, testes, and egg yolks of chickens artificially infected with the virus from pseudo fowl pest chickens failed in protecting susceptible chickens from infection, resulting in the lack of immunity against natural viral infections and death [37].

An experiment in 1938 was conducted by injecting 25 pseudo-fowl pest virus strains obtained from Java, Sulawesi, and Sumatra provinces into mice. The virus was injected through the intracerebral route with a “maximal tolerable amount” (0.05 mL of brain suspension in sterile physiological saline solution 1:10, of which 1 mL of solution from 1:100,000 is fatal in chickens). On the other hand, those mice did not show clinical symptoms and appeared healthy. On the other hand, mice were infected when injected with virus strains obtained from the Netherlands and Switzerland through intracerebral injections [38].

The study continued from the seed virus obtained from NDV-infected chickens in Jakarta in 1936. On the other hand, after World War II ended, the virus used for the vaccines had died, and vaccines made from other seeds did not provide satisfactory results. Because the outbreak occurred around Jakarta, vaccine development was re-developed in 1948. Furthermore, vaccination experiments were carried out using seed vaccine viruses from Bogor. Nevertheless, chicken deaths still occur [33].

Suminta vaccine strain

Martini and Koerjana [39] then isolated several strains of the NDV from the outbreak and obtained one strain that was considered the best, namely the “Suminta” strain. Experimentally, the clinical signs first appeared 34–48 h post-infection, and death occurred after an average of 24 h post-infection. In the dead birds, hemorrhagic lesions were significant, characterized by necrotic regions of the large intestine (CRAWEORD’s lesions) [39]. The first Indonesian NDV strain was propagated using embryonated chicken egg (ECE) media. Serial injected virus in ECE resulted in a high virus concentration in the allantoic fluid, up to 20×10^{-17} MLD at 1 cm^3, and the Hirst titer was 1:640 or more. These results then led to further research on attenuated strains of the NDV as a first step in preparing an efficient vaccine [39].

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On November 15, 1949, the first exploratory vaccination test was started with the pseudovogelpest vaccine prepared by the Institute for Animal Diseases (now referred to as the Indonesian Research Center for Veterinary Science, IRCVS), which proved that this vaccine could boost immunity. The vaccine used a strain from Manilla that had been refrigerated for eight months, resulting in low virulence in chickens. Vaccination was applied to 18,929 chickens in several cities in Indonesia, e.g., Surabaya, Bandung, Jakarta, Purwakarta, Serang, and Bogor. The number of cases of paresis, paralysis, and mortality was insignificant, with a very low mortality rate (2%) and no cases of NDV infection in vaccinated chickens. On the other hand, egg production was reported to decrease significantly in purebred chickens. Hence, vaccination was not recommended for chickens in the production period [40].

Furthermore, isolation of the "Suminta" strain encouraged further research in obtaining less virulent strains through intracerebral inoculation from various animals to use the attenuated viruses for poultry immunization. Two strains of the highly pathogenic Indonesian NDV strain "Suminta I" could be attenuated by an intracerebral re-injection in ducks (Suminta I E.K strain) and in cooli-coo birds (Suminta I Perkutut strain). The virulence of these two strains in the nervous system was much less than the two strains of Mukhteswar. When injected intramuscularly at a dose of 30 hemagglutinin unit (HU) for Suminta I Perkutut and 10 HU for Suminta I E.K., both strains induced a stronger immune response than the Mukhteswar strains (Manila dan Cairo). These results suggest that the Suminta strains are practically apathogenic when applied intramuscularly and have met the requirements of a good viral vaccine [41].

A publication in 1952 reported many deaths of chicks showing a loss of appetite, lethargy, difficulty breathing, paralysis, and death after 2–3 days of the post-clinical signs. The previously developed vaccination of chicks resulted in death, but the petechiae clinical signs in proventriculus were not found. This shows that vaccination in chicks may result in insufficient immunity. Thus, revaccination is necessary [42]. Furthermore, Djaenoedin and Koerjana [43] reported that the vaccine made by the IRCVS from Indonesian isolates was excellent and strong. This statement was based on the administration of the vaccines to chickens, which were then challenged with a pathogenic NDV. The feces from vaccinated chickens were injected and administered orally to unvaccinated chickens; none died [43]. **Table 2** lists the pseudo fowl pest vaccine doses used in poultry in 1950–1957 in Indonesia.

Ronohardjo [44] highlighted the maternal antibodies acquired by chicks from mothers infected with the NDV. This immunity can prevent the formation of an active immune system against natural NDV infections and immunity through vaccination. He reported that the maternal antibodies acquired by the chicks through the vaccinated hens decreased in titer after the chicks reached 4–6 weeks [44]. Although this immunity cannot prevent chicks from natural ND infections entirely, it produces an infection with subclinical symptoms. A follow-

Table 2. The doses of pseudo fowl pest vaccine used in poultry in 1950–1957 in Indonesia

Year	Vaccine doses (doses)
1950	1,589,500
1951	4,908,920
1952	10,250,250
1953	13,414,250
1954	13,703,000
1955	13,401,750
1956	9,619,250
1957	8,307,500

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up study by Ronohardjo [45] reported that maternal antibodies in chicks determine the length of the incubation period, mortality, and morbidity. When challenged with a field virus, chicks with high maternal antibody titers had a more extended incubation period and death time and lower morbidity and mortality rates than those with lower titers. Higher titers cause chicks to be more resistant to high doses of challenge virus. In contrast, the challenge virus wiped out all the six-week-old chicks with no maternal antibodies [45].

La Sota and Komarov seed vaccine

Ronohardjo [46] compared several NDV-isolated antigens with the La Sota and Komarov strains using the Agar Gel Diffusion technique. The study showed that 10 isolates obtained from West Sumatra, Yogyakarta, Cirebon, Bogor City, and Bogor Regency, which had been purified from allantoic fluid with $BaSO_4$ and sodium citrate, were identical to the NDV from the La Sota and Komarov [46]. Vaccination development was continued using a combination of F and Komarof vaccine strains in 1977. Previously, one study proved that using the Komarov virus strain in the ND vaccine could provide high titer and long-lasting immunity compared to the vaccine with lentogenic NDV (La Sota, F, and B1). Therefore, Ronohardjo et al. [47] combined the nasal drip vaccine of the NDV F with a vaccine injection of the NDV Komarov by administering three drip vaccinations at two weeks old, 4–5 weeks, and 12–14 weeks for the intramuscular injection vaccine. The average titer result showed that the post-vaccinated chicks were sufficient to prevent a possible outbreak of the NDV [47].

The ND outbreak still occurred and could not be eradicated, even though there were enough vaccines on the market at that time. Thus, Ronohardjo et al. [48] tested the potential for the ND vaccine circulating in the market obtained from producers, importers, distributors, and sellers in Bandung, Jakarta, and Bogor. They reported that not all vaccines on the market met the optimal standards for the vaccine [48]. At that time, the use of active vaccines in laying hens had been widely practiced because they produced sufficient immunity compared to inactivated vaccines. On the other hand, La Sota and Komarov active vaccines were not recommended for chickens during production because they could temporarily reduce egg production. To that end, Ronohardjo et al. [49] conducted a study related to the immunization of laying hens with an inactivated ND vaccine in oil adjuvant (Imopest). The Imopest vaccine was safe to use, had no effect on egg production, and could produce more stable and long-lasting immunity than the active vaccine [49].

Research Institute for Veterinary Science (RIVS) seed vaccine

Further vaccine research was conducted on free-range chickens. In 1975, the lentogenic strain of NDV isolated in 1967 was resistant to temperature and was considered excellent as an ND vaccine (V4.NDV). V4.NDV was then imported to Indonesia from Australia through the Research Institute for Veterinary Science (RIVS/BB LITVET), Bogor. Research into the stability of V4.NDV was carried out at several temperatures in 1985. A live ND vaccine was developed from this variant. For small-scale farmers who generally live in rural and transmigration areas, the RIVS V4.NDV variant (stable at high temperatures and given through rice) was considered simple to apply and was expected to protect chickens from the NDV. Under a laboratory environment, the RIVS V4.NDV vaccine in free-range chickens, administered orally, protected against the velogenic Ita NDV strain. The resulting immunity increased from 50% to 78% when a booster was applied four weeks later [50].

Darminto et al. [51] reported a field trial of an oral ND vaccine administered through the grain in the province of Riau in 1990. Chickens receiving oral ND vaccination had varying

protection against the NDV, depending on the type of vaccine used. The RIVS2 vaccine provides 70% protection with 96.1% protection against ND outbreaks in the field, while the RIVS3 vaccine provides 62.5% immunity with 72.3% protection. RIVS2 chickens boosted with the RIVS4 vaccine had 58% immunity, 6% each in the artificial-infected test and outbreaks in the field [51]. In addition to Riau Province, RIVS4 vaccine testing was also carried out in Bogor Regency. The vaccine with a titer of 100×10^7 EID_{50}/mL was dissolved in clean water until 10^7 EID_{50}/mL and mixed with 700 g of grain, which was then distributed to 100 free-range chickens. The vaccinated chickens had a lower mortality (2.81%–23.28%) than the unvaccinated chickens (79.26%). The RIVS4 vaccine became an alternative to deal with the NDV in extensive free-range chickens [52].

CHALLENGE TO CONTROL ND CASES

Virus distribution

A study in Denpasar and Kupang in 1989–1990 showed a higher distribution of virulent NDVs than avirulent NDVs in the environment, in which the highest prevalence was in September and October 1989, and the lowest was in April and July 1990. On the other hand, in Irian Jaya, an avirulent NDV strain was distributed more widely in the environment than the virulent NDV, except for two cases that occurred in September 1989 (Merauke) and 1990 (Jayapura). Based on the virus characterization with monoclonal antibodies, the isolates obtained during the study did not have antigenic similarity to the RIVS.V4 strain but had similar biological properties [53]. An epidemiological study in eastern Indonesia revealed 126 NDV isolates from several bird species [54]. Most of the isolates (97.6%) belonged to the malignant (velogenic) strain, while the other three isolates (2.4%) belonged to the mesogenic strain.

Development of vaccines for rural areas

Several items need to be considered when choosing the most effective and practical vaccination program: 1) required level of protection, 2) poultry immune status, 3) local NDV pathogenicity, 4) correlation with other poultry diseases, 5) ND vaccine application, and 6) monitoring the immune response of the flock [55]. Further research on vaccination efficiency was carried out on 1,000 broiler chickens, suggesting that the RIVS4 vaccine had excellent lateral transmission. This was indicated by the antibody and protective response of indirect-vaccinated chickens, showing the same results as the direct-vaccinated chickens [56].

Although various types of vaccines were available, implementing ND vaccination in extensively reared free-range chickens still faces technical difficulties in the field. Therefore, a new approach was needed to control ND in those chickens. On the laboratory scale, the RIVS2 and RIVS3 vaccines given with small-grain rice or aron rice with two vaccinations (three-week intervals) could protect against the NDV, and the protection would increase if added with a booster four weeks after the second vaccination. On the other hand, the results were quite varied in the field scale. This was attributed to the influence of antimicrobial substances, including virucidal substances naturally found in grain skins. Thus, another alternative was administering a vaccine with aron rice as a substitute for grains [57].

Further research was conducted on the resistance of four oral ND vaccine strains on several types of chicken feed, namely small raw rice grain, small raw rice grain boiled for 10 minutes and dried, rice grain washed three times, dried-chopped cassava grains, raw white rice, cooked aron rice, cooked white rice, washed large raw rice grain, and large raw rice grain

boiled for 10 minutes. The virus could not be detected 0 hours after mixing with white rice. After six hours of mixing, the virus could not be detected in the small and large grain raw rice carriers. The virus could still be detected in other carrier materials after 24 hours of mixing, even though the titer was decreased [58].

Development of vaccines according to circulating viruses

Indonesia is an endemic country for ND where the field strain velogenic NDV circulates. There was no inactivated vaccine containing velogenic NDV from the field in 1996. As the cause of the ND outbreak was a field virus, a field-isolated ND vaccine is needed for higher effectiveness and efficacy. Velogenic NDV, Ita strain, was used to prepare the inactivated antigen for further evaluation. The inactivation of velogenic NDV with formalin was quite effective, but the inactivated antigen was more stable when using an inactivation temperature of 4°C compared to room temperature (37°C). The velogenic NDV antigen inactivated with formalin at 4°C and emulsified with adjuvants, both lanoline-paraffin and aluminum hydroxide, was still immunogenic. Hence, they have good prospects to be developed as an inactivated ND vaccine for local isolates of velogenic strains [59]. In further testing, the inactivated velogenic ND Ita strain vaccine emulsified with lanoline-paraffin and aluminum hydroxide adjuvants could protect chickens from illness/death and decrease egg production due to NDV infection [60].

Indonesia has a risk of NDV infection, which can threaten poultry health and cause economic losses to farmers. Therefore, an intensive vaccination program is needed. Experiments in the laboratory and field showed that two vaccinations carried out at four days of age and repeated at 14–21 days old were satisfactory in protecting commercial broiler chickens [54,61]. Based on lateral transmission, ND vaccination by eye drops in 33% of the population was sufficient to increase chicken immunity against the NDV with a density of 10 birds/m^2, and vaccination was carried out twice, at four and 14 days old. Approximately 70% of other unvaccinated chickens will acquire immunity through lateral transmission in a short time [61]. In free-range chickens, the lateral vaccination transmission system only works for those kept intensively in closed cages [62].

The impact of the vaccination program with heat-resistant ND vaccine (RIVS2) and local velogenic NDV (Ita strain) isolates on free-range chickens was analyzed. The study stated that the RIVS2—Ita vaccination program showed no significant difference from the commercially active and inactivated vaccines. Therefore, the combination of IRCVS vaccines has the same ability to increase ND antibody titers in chickens as commercial vaccines. By contrast, the vaccination with active RIVS2 showed a significant difference in increasing antibody titers compared to commercial active vaccines. The active RIVS2 vaccine appeared to have greater ability to increase the antibody titers after the first vaccination than the commercial ones [63]. An effective poultry disease control program requires a combination of strict biosecurity measures with vaccination. A promising NDV vaccine can prevent clinical disease, reduce or eliminate virus shedding, and reduce the lethal virus. Vaccination research in Indonesia was developed using various strains, as discussed above.

VARIABILITY OF NDV AND ITS CONTROL

Virus diagnosis and identification

Virus identification in poultry must be done quickly to achieve appropriate and immediate prevention and control measures. Hence, a good diagnostic method is needed, considering that

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the NDV is endemic in all regions in Indonesia. The NDV can be detected through molecular and serological tests. The serological test for NDV involved using the hemagglutination inhibition (HI) and enzyme-linked immunosorbent assay (ELISA) test methods.

Monitoring the immune status of a chicken farm is very important for evaluating the vaccination results. Hence, this monitoring activity should be integral to overall ND disease control. The development of monoclonal antibodies against the NDV was reported using the ITA p13 (velogenic viscerotropic) strain harvested from the allantoic fluid of nine-day-old SPF eggs in 1991. The antibody produced did not cross-react with the ND lentogenic strain and showed positive results for the velogenic strain. Thus, the monoclonal antibody developed can differentiate between velogenic and lentogenic NDVs [64].

Parede and Indriani [65] developed another monoclonal antibody based on the method used by the Central Veterinary Laboratory, Weybridge, England. The developed monoclonal antibody belongs to IgG2a, based on mouse isotyping tests. Six monoclonal antibodies have been successfully developed to differentiate velogenic and non-velogenic NDVs based on the presence or absence of reactions [65].

When a high-mortality disease outbreak occurs, diagnostic capabilities are needed to determine the cause, whether by the NDV or other viruses with similar clinical signs. Nevertheless, more research is needed to develop an accurate identification for ND. Indirect detection and capture ELISA detection methods were developed to differentiate the NDV virulence. The two diagnostic tools identified the pathotype of the NDV [66]. In addition, a competitive ELISA (C-ELISA) was developed using two monoclonal antibodies that react specifically with the epitope on the HN protein (anti-HN) and NP protein (anti-NP). Compared with indirect-ELISA and HI assays, C-ELISA is more sensitive for detecting NDV antibodies. It detected seroconversion faster with a higher percentage [67].

The IRCVS has produced dry, thermostable antigens to fulfill the need for antigen NDV for serological testing. Dried ND antigens added with a TR-18 formulation using the freeze-dry and Xerovac methods can produce stable products without lowering the titer. The formulation with TR-18 can protect the antigen from degradation because of the lower temperature and drying process [68]. A reverse transcription polymerase chain reaction (RT-PCR) test was then developed to detect the NDV genome in clinical samples owing to its high sensitivity and accuracy. Therefore, molecular-based pathotyping is a good alternative to conventional virus isolation, which is considered slow and requires special facilities. Hartawan and Dharmayanti [69] developed the multiplex RT-PCR test against ND, avian influenza, and infectious bronchitis viruses that have similar clinical symptoms. The three viruses could be detected using the test. Nevertheless, regular primer development may be needed based on the evolution of the NDV.

In addition to affecting the detection ability of diagnostic tools, NDV mutations can cause the failure of the vaccination program. New exotic strains can also affect the efficacy of the ND vaccine used. In Indonesia, infection with the genotype VII NDV has been reported to cause outbreaks in several poultry farms. Consequently, molecular characterization of the NDV is needed to assess possible vaccine candidates.

Virus diversity

In Bali, disease outbreaks in free-range chickens were reported with symptoms of depression, anorexia, dyspnea, torticollis, dull feathers, paralysis, and death. The clinical signs and

infectivity lead to the NDV. Based on molecular studies, the NDV/Bali-1/07 virus isolate has a cleavage site motif of 112RRQKRF117 on protein F, which causes this isolate to be included in the virulent NDV. Unlike the La Sota strain included in group II, the NDV/Bali-1/07 virus isolate belongs to group VII, which is the dominant group in Asia. NDV/Bali-1/07 virus has a nucleotide homology with group VII NDV in Indonesia, such as Cockatoo/Indonesia/90 by 96% [70].

Dharmayanti et al. [4] reported six NDV isolates from West Java, Banten, and East Java in NDV genotype VII, one isolate in genotype VI, and another in genotype I. Based on its F gene, seven isolates had the R-R-R-K-R and R-R-Q-K-R sequences, which are markers of the velogenic NDV pathotype. One isolate (RIVS) had the G-K-Q-G-R-L sequence, which is the pathotype of the lentogenic NDV [4].

Genotype VII NDV was also isolated from live poultry markets in several districts in West Java, including Bogor, Sukabumi, and Tangerang. Indonesia has a mixed poultry production system, in which most poultry are raised traditionally, and many have markets selling live birds of various species and ages. Historically, traditional farms and live poultry markets were considered the main point in the evolution of velogenic strains of the NDV because of the difficulty of vaccinating newly hatched birds. Unvaccinated commercial and domestic poultry, as well as wild birds, may act as reservoirs of the NDV, transmitting it to susceptible birds [34]. **Fig. 4** presents an overview of the activities that occur in the live animal market. The NDV sequences isolated at West Java in 2013 and 2015 were reported in 2020. The ITA strain (chicken/Indonesia/ITA/012WJ/1951) was grouped as the genotype VI NDV with a cleavage site on the protein F 112R-R-Q-K-R-F117.

Fig. 4. Activities in one of the live poultry markets in Indonesia are considered the cause of the evolution of the velogenic strain of NDV. (A, B) Interactions between birds of the same species originating from different locations and contact that occurs between birds and people around without using proper protection. (C, D) Interactions that occur between different species of birds that have different ages.
NDV, Newcastle disease virus.

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The Cilebut Chicken/Indonesia/Cilebut/010WJ/2015 strain isolated in 2015 belongs to genotype VII with the 112R-R-Q-K-R-F117 sequence [71]. By contrast, based on the F gene, chicken/Indonesia/Mega/001WJ/2013 and chicken/Indonesia/Cimanglid/002WJ/2015 virus strains belong to the NDV genotype VII.2 in class II avian paramyxovirus, each having a 112RRQKRF117 and 112RRRKRF117 sequence [72]. Data from the Indonesian Ministry of Agriculture via iSIKHNAS showed that the NDV still infects poultry populations in several provinces in Indonesia. In 2020, 8,060 birds were affected by the NDV, while in 2021 and 2022, the number of NDV-infected birds decreased, with 7,413 and 530 affected birds, respectively (**Fig. 5**).

The virulent and avirulent strains of NDV have been circulating among vaccinated chickens in West Java, Indonesia, as indicated by the finding of NDV isolates in West Java in vaccinated poultry in 2011, 2014, and 2015 [73]. A recent study conducted in South Sulawesi also showed that ten NDVs isolated from commercial poultry farms in 2019 had a virulent pathotype marker with the 112R-R-Q-K-R-F117 sequence in the cleavage site protein F. Based on a phylogenetic study of the partial F gene, this virus has 87.82%–97.96% identity with the NDV genotype/subgenotype VII.2 compared to other subgenotypes in Indonesia. The studies above showed that genotype VII is the predominant NDV circulating in the field. Hence, it is necessary to update the master seeds to obtain a more effective vaccine. The NDV outbreaks detected in vaccinated poultry farms indicate that the vaccination was ineffective. Thus, it is necessary to improve the current NDV control strategy. One measure that can be used is to develop a master seed of the NDV vaccine based on the circulating virus in the field [74].

Four NDVs that have different genotypes were used as vaccine seeds against the Indonesian NDV/GTT/11 genotypes VII in chickens: Chicken/Indonesia/GTT/11 genotype VII, Chicken/Indonesia/I/53 genotype VI, Indonesia/RIVS genotype I (BBLitvet), and Lasota (commercial) genotype H. These genotypes provided 100% protection from ND disease symptoms and death. On the other hand, the virus excretion in chickens vaccinated with genotype VII ND was significantly different ($p < 0.05$) compared to other groups. Chickens vaccinated with genotype VII also showed a higher average titer (GMT 122) than chickens vaccinated with other vaccines. Therefore, ND chicken/Indonesia/GTT/11 virus genotype VII can be used as a seed for inactivated ND vaccine and is compatible with the virus circulating in the field [75,76].

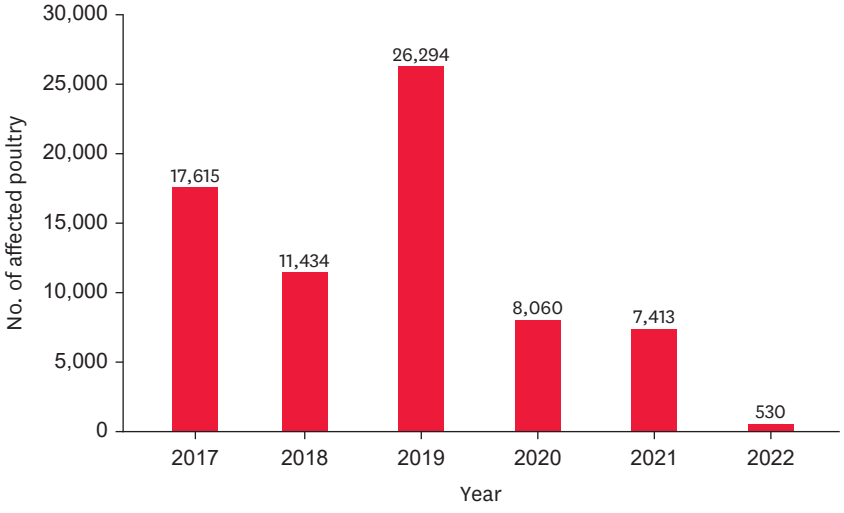

Fig. 5. Overview of the distribution of ND in affected poultry in the last five years in Indonesia.
ND, Newcastle disease.

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On a laboratory scale, the Indonesian ND/GTT/11 genotype VII inactivated vaccine, which contains 256 HAU antigens for one dose, provides an excellent post-vaccination response with an average titer of 7.3 log 2 after two weeks of vaccination and can provide 100% protection against the clinical symptoms in chickens. The vaccine can be an alternative to ND vaccination [75,76]. The compatibility between the vaccine and circulating virus isolates is essential for better protection against virus transmission by reducing the magnitude of viral shedding. Nevertheless, further research will be needed to update vaccines to control the circulating NDV in the field.

Various vaccine developments have been carried out in Indonesia, starting with various methods with isolates found in Indonesia, the use of the Suminta Vaccine Strain, the use of other vaccine strains, namely La Sota and Komarov seed vaccine and the use of the RIVS4 vaccine developed by a veterinary research institute. Various strains are circulating in Indonesia, with the most recently isolated genotype being genotype VII. Among them, four NDVs with different genotypes were used as vaccine seeds against the Indonesian NDV: Chicken/Indonesia/GTT/11 genotype VII, Chicken/Indonesia/I/53 genotype VI, Indonesia/RIVS genotype I (BBLitvet), and Lasota (commercial) genotype H. Although various vaccine developments have been carried out, several outbreaks still occur in Indonesia, requiring further research on the virus epidemiology and vaccine development. Nevertheless, vaccine development in Indonesia is still focused on vaccines circulating in the field.

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