Newcastle disease (ND) is endemic in Indonesia and other countries. It is a highly contagious and fatal viral disease affecting most species of birds in which chickens are the mostly susceptible. The disease is frequently responsible for devastating losses in poultry. NDV strains can be classified as highly virulent (velogenic), intermediate (mesogenic) or non-virulent (lentogenic). This classification is based on the results of the mean death time in chicken eggs. The clinical signs of a highly virulent NDV infection in chickens can be extremely different depending on the strain of virus.

The major clinical signs of ND caused by a velogenic NDV infection in chickens are severe diarrhea, dehydration, dyspnea, and neurological manifestations such as torticollis and paralysis. Velogenic NDVs are classified based on their pathotypes as viscerotropic velogenic (VV-) NDV and neurotropic velogenic (NV-) NDV. VV-NDVs, which cause diarrhea and frequently hemorrhagic intestinal lesions, are found in most of the countries endemic of NDV. NV-NDVs, which cause respiratory and neuronal lesions such as paralysis, twitching, and torticollis without inducing obvious enteritis, are only isolated in United States. Interestingly, neurotropic lesions were often reported recently in chickens infected with VV-NDV. The mechanism of the alternative encephalitis found in chickens infected with VV-NDVs is not yet clearly understood although some implications such as ineffective vaccinations have been discussed.

NDV has high viral diversity as at least 8 genotypes (I to VIII) are recognized based on phylogenetic analysis of fusion (F) gene sequence. NDVs in genotypes VII are also called as Asian type, since they are major devastating NDV strain recently found in Asian countries including Indonesia. The NDV is an enveloped virus belonging to paramyxoviridae with a negative-sense, single-stranded, non-segmented RNA genome. In general, negative-stranded RNA viruses, especially non-segmented negative-sense RNA viruses, have lower rates of recombination than positive-stranded RNA viruses such as polio virus. However, recently the evidence of emergence of new NDV strain through recombination of virulence circulating strain with LaSota strain, which is widely used as live vaccine, has been reported.
As the ND case in the field still not overcome yet even vaccination program has been done for many decade. Unvaccinated birds and “incomplete” vaccination might be the major causes of this situation. However, the generation of chimeric virus or mutated viruses, as well as the immunological status of the host birds would also affect the pathology of NDVs. For the understanding of ND, it would be important to study the pathology of recent generation of NDVs using regional host chickens. Unfortunately, there is quite few NDV isolates from Southeast Asia with defined complete genome sequences. Thus, in this study, the isolation of a novel pathogenic NDV from a local outbreak of ND in Indonesia (Chapter 1), the analysis of the pathogenicity of the isolated virus to the locally used layer chickens (Chapter 2), the complete genome sequence determination (Chapter 3) were performed. Reservoir bird species such as water fowls are also the reason for NDV endemic. Thus, serological survey of NDV in Bali ducks which are commonly kept close to chickens in traditional farming system in Bali (Chapter 4).

I. Isolation and characterization of a pathogenic Newcastle disease virus from a natural case in Indonesia

This study was performed to isolate a velogenic Newcastle disease virus (NDV) strain currently found in Indonesia for establishing a domestic reference virus for future pathological and molecular epidemiological studies. A chicken suspected to have contracted ND in a local outbreak in Bali was selected for NDV isolation. Atrophy of lymphoid tissues such as the bursa of Fabricius, thymus, and spleen; intestinal hemorrhage; and edema of the brain were observed in the chicken. Histopathological examination revealed severe non-suppurative meningoencephalo-myelitis characterized by neuronal necrosis, multifocal to diffuse gliosis, and perivascular cuffing of mononuclear cells, hemorrhagic necrosis of the trachea, intestines and bursa of Fabricius, and various degree of lymphoid depletion and necrosis of the lymphoid tissues. After ND was confirmed immunohistochemically, the NDV was propagated by inoculating tissue homogenate of the diseased chicken into embryonated eggs. Phylogenetic analysis based on the F gene nucleotide sequence revealed that this isolate belonged to genotype VII, which includes newly panzootic Asian strains. The deduced amino acid sequence of the isolated NDV F protein at the cleavage site was RRQKRF, which is typically found in virulent NDV isolates. Pathogenicity indexes such as the
mean death time (MDT) and intracerebral pathogenicity index (ICPI) were 54 hr and 1.77, respectively, indicating the isolated virus is a pathogenic NDV. Pathological findings, phylogenetic analysis, amino acid sequence of the F protein cleavage site, and pathogenicity index test results revealed the NDV isolate, designated as NDV/ Bali-1/07, to be a novel Indonesian velogenic NDV strain belonging to the genotype VII.

II. Pathogenicity study of NDV/Bali-1/07 in 3- and 8-week-old ISA brown chickens and protectivity of LaSota vaccine againsts the Bali-1/07

In this chapter, the pathogenesis of NDV/Bali-1/07 to the ISA/brown chicken, a popularly used layer chicken in Indonesia, was studied. Antibodies are known to alter or enhance the infection of viruses. Maternal antibodies could modify the pathogenicity of NDV/Bali-1/07. Thus, serum maternal antibody levels were monitored from 1-day old chicks. At 3-week-old, maternal antibody titers decrease to \(< 2^6\), which is under the suggested protective level. No maternal anti-NDV antibody (HI titer) was detected at 8-week-old. Thus, 3- and 8-week-old chickens were inoculated with \(10^5\) TCID\(_{50}\) NDV/Bali-1/07. Another group of chickens were vaccinated at 3- and 6-week-old with commercial LaSota vaccine that is commonly used in Indonesia and infected with NDV/Bali-1/07 similarly. All chickens infected at 8-week-old showed viscerotropic clinical signs such as diarrhea and all of them died within 5 days post infection (dpi). None of them showed clear neuronal signs before died, indicating that the NDV/Bali-1/07 is a viscerotropic velogenic NDV strain. The infected 3-week-old chickens also showed viscerotropic clinical signs mostly very slightly. Interestingly, all of the chickens recovered from enteritis exhibited neuronal signs at 8 dpi. Survival rate in this group was 60%. These results suggest the possibility of the contribution of maternal antibodies to the development of neuronal signs. No clinical signs were observed and no chicken died in vaccinated group, suggesting that the LaSota vaccine (genotype II) can induce protective immunity to NDV/Bali-1/07.

III. Complete nucleotide sequence of NDV/Bali-1/07 and characterization of each gene

The complete genome sequence of NDV/Bali-1 was determined and its genetic relationship to other NDV strains was studied by phylogenetic analyses. Complete genome size of the NDV/Bali-1/07 was 15,192 nucleotides (nt), which was 6-nt longer than that of NDV LaSota live vaccine strain used in Indonesia. The genome consists of 6 genes in the order of 3'-N-P-M-F-HN-L-5' and contains a 55-nt leader region at the 3'-end and a 114-nt trailer region at the 5'-end. The nucleotide sequence identity of the entire viral genome of Bali-1/07 to other NDV strain within Class II ranged between 82.4 and 93%, whereas to that of Class I NDV was 71.6 %. Phylogenetic analysis of the fusion (F) gene of NDV/Bali-1/07
revealed that the NDV/Bali-1/07 belongs to genotype VII subtype a (VIIa). NDV/Bali-1/07 was always grouped with other members of NDV genotype VII in phylogenetic analyses based on the non-coding sequences and/or gene regions other than F gene, suggesting that NDV/Bali-1/07 has acquired no recombination with NDVs of other genotypes.

IV. NDV infectivity in Bali Ducks

Almost all of the poultry species are susceptible to the infection resulting from the ND; however, chicken are the most susceptible. Waterfowls such as, ducks and geese, are considered potential reservoirs of NDV. In general, duck species may show few or no clinical signs even for NDV strains lethal to chickens, yet they are capable of harboring the virus. There is no report about seroprevalence of ND in the other species of poultry, such as Bali ducks, although many reports have demonstrated ND cases in chicken. Hence sero-epidemiological study on NDV in Bali ducks has been conducted. A total of 119 samples of duck sera was collected from traditional farming system at Sangeh and Carangsari, the villages located in Badung Regency, and at Padangsambian, a village located in Denpasar and examined using ELISA to identify the presence of anti-NDV antibody. It could be concluded that the Bali ducks could be infected by the ND virus with total seroprevalence of 91.6%. The seroprevalence in young duck and old duck were 86.4% and 92.8%, respectively. Although the seroprevalence of NDV antibody in old ducks was higher than that of young duck, there was no significant differences (p>0.01) statistically between the young and old ducks. From the research findings, it could be concluded that the Bali duck could be infected by the NDV and that the Bali ducks could potentially transmit it to other susceptible animals including chickens.

Conclusion: The newly isolated NDV/Bali-1/07 was VV-NDV belonged to genotype VII subgenotype VIIa, with no recombination with other genotypes. NDV/Bali-1/07 might be useful for pathological studies on ND as one of the reference NDV of Southeast Asia-origin. Furthermore, NDV/Bali-1/07 could be used for further analyses such as chimeric virus production and pathological assays, since whole genome sequence was obtained. It is also suggested that the Bali ducks are infected by NDV and are potential reservoir of NDV in Bali. They might be a source of NDV which cause outbreaks of ND in chickens.