

Isolation and Identification of Newcastle Disease and Avian Influenza Viruses in Commercial Chickens From Marga and Penebel Districts of Bali

(ISOLASI DAN IDENTIFIKASI VIRUS TETELO
DAN FLU BURUNG DARI AYAM RAS
DI KECAMATAN MARGA DAN PENEHEL, BALI)

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ABSTACT

Newcastle Disease and Avian Influenza subtype H5N1 are highly contagious poultry diseases that cause substantial economic losses in the poultry farming industry. This study was aimed to identify the infections of Newcastle Disease and Avian Influenza viruses in commercial chickens in Marga and Penebel Districts, Tabanan Regency, Bali Province, by observing clinical signs, anatomical pathology changes and performing hemagglutination (HA) and hemagglutination inhibition (HI) tests. The research involved 16 broiler chickens samples showing suspected signs of Newcastle Disease and Avian Influenza H5N1 infections. The methods included field clinical examination (observation), pathological examination (necropsy) to assess anatomical pathology, virus isolation using embryonated chicken eggs, and serological testing (HA and HI tests). The findings indicated that chickens infected with Newcastle Disease and Avian Influenza generally exhibited greenish-white diarrhoea, respiratory disorders such as rales, coughing, and difficulty in breathing, as well as neurological signs including torticollis and incoordination. Anatomical pathology findings revealed haemorrhages in the lungs, intestines, and cecal tonsils, petechiae on the proventriculus, and brain congestion. Of the 16 broiler chicken samples, ten tested positive in the haemagglutination test, while five were confirmed by haemagglutination inhibition as infected by Newcastle Disease and or Avian Influenza H5N1. The research results concluded that the combination of clinical examination, pathological examination (necropsy) and HA and HI laboratory tests was effective in determining a definitive diagnosis of ND and AI.

Keywords: Newcastle disease subtype H5N1; Avian Influenza; commercial chickens;
HA/HI test

ABSTRAK

Peyakit tetelo (*Newcastle Disease*) dan flu burung (*Avian Influenza*) sub tipe H5N1 adalah penyakit unggas yang sangat menular dan menyebabkan kerugian ekonomi yang besar pada industri peternakan unggas. Penelitian ini bertujuan mengidentifikasi infeksi virus penyakit tetelo dan flu burung tersebut pada peternakan ayam di Kecamatan Marga dan Penebel, Kabupaten Tabanan, Provinsi Bali, dengan mengamati tanda klinis, perubahan patologi anatomi, serta melakukan uji hemaglutinasi dan hambatan hemaglutinasi. Penelitian menggunakan 16 ekor sampel ayam pedaging yang menunjukkan tanda-tanda kuat terinfeksi penyakit tetelo (*Newcastle Disease*) dan flu burung (*Avian Influenza*) sub tipe H5N1. Metode yang digunakan meliputi observasi lapangan, nekropsi cadaver ayam untuk mengamati perubahan patologi anatomi, isolasi virus menggunakan telur ayam yang telah berembrio dan uji serologi. Hasil penelitian menunjukkan bahwa ayam yang terinfeksi penyakit tetelo dan flu burung umumnya menunjukkan gejala diare berwarna putih kehijauan, gangguan pernapasan seperti *rales*, batuk dan kesulitan bernapas, serta tanda neurologis termasuk tortikolis dan inkoordinasi. Temuan patologi anatomi memperlihatkan perdarahan pada paru-paru, usus dan tonsil sekum, petekie pada lambung kelenjar atau proventrikulus, dan pembendungan (kongesti) pembuluh darah otak. Dari 16 ekor ayam sampel, sepuluh di antaranya positif berdasarkan uji hemaglutinasi (HA), sementara lima sampel diteguhkan berdasarkan uji hambatan hemaglutinasi (HI) terinfeksi oleh penyakit tetelo dan atau oleh flu burung sub tipe H5N1. Hasil riset disimpulkan bahwa kombinasi pemeriksaan klinis, pemeriksaan patologi (nekropsi) dan uji laboratorium HA dan HI efektif untuk menentukan diagnosis definitif ND dan AI.

Kata-kata kunci: penyakit tetelo (*Newcastle Disease*); flu burung (*Avian Influenza*) sub tipe H5N1; peternakan ayam; uji HA/HI

INTRODUCTION

Newcastle Disease is a highly contagious viral infection in poultry caused by Avian Paramyxovirus serotype 1, belonging to the genus *Avulavirus* and family Paramyxoviridae (MacLachlan and Dubovi, 2016). Its severity varies from subclinical infection to severe disease with mortality rates up to 100% (OIE, 2012). Meanwhile Avian Influenza is caused by a virus belonging to the Orthomyxoviridae family with a segmented RNA genome that promotes mutation (Lycett *et al.*, 2019). Both diseases present similar clinical signs, including respiratory, digestive and nervous disorders, such as greenish-white diarrhoea, coughing, sneezing, torticollis and incoordination (Kencana *et al.*, 2012; Ayuti *et al.*, 2024). These viruses spread rapidly through faeces and respiratory secretions, contaminating the environment -

(OIE, 2021). In Bali, especially Tabanan Regency, Newcastle Disease and Avian Influenza remain endemic and cause significant losses (Kencana *et al.*, 2012; Kencana *et al.*, 2021). However, most available data in this region are based on historical outbreaks, leaving a significant gap in understanding the current antigenic characteristics of the circulating viruses. Given the high mutation rate of Avian Influenza and the persistent nature of Newcastle Disease, there is an urgent need to re-evaluate the field strains to ensure that existing control measures and vaccination programs remain effective against contemporary threats. This study was aimed to isolate Newcastle Disease and Avian Influenza viruses from field cases using embryonated chicken eggs and identify them through hemagglutination and hemagglutination inhibition tests (Emilia *et al.*, 2015; OIE, 2021), as part of the efforts to

improve poultry disease control strategies in the region.

RESEARCH METHODS

Research Object

This study is descriptive observational which the object of this study was 16 head of commercial broiler chickens age 2-3 weeks with a presumptive diagnosis of Newcastle Disease. Eight chickens originated from Marga District and eight from Penebel District, Tabanan Regency, Bali. To minimize selection bias, the inclusion criteria were strictly defined as chickens exhibiting a clinical score of at least based on pathognomonic signs, including respiratory distress (gasping), digestive disorders (greenish diarrhoea), and neurological symptoms (torticolis or incoordination) and were sampled directly from farms in both districts.

Research Materials

Samples consisted of organs showing lesions compatible with Newcastle Disease and Avian Influenza infections, including whole brain, apical lobes of the lungs, proventriculus, duodenal loop and cecal tonsils. Sample collection was performed through field surveys, followed by necropsy after the death of the chickens using standard dissection tools (scalpel, scissors, forceps) and collection containers (Eppendorf tubes, organ containers) to collect approximately 2–5 g of each target tissue. Samples were transported in cool boxes and either processed immediately or stored at -20°C . Tissue suspensions (10–20%) were prepared in phosphate-buffered saline (PBS), homogenized with sterile instruments, centrifuged at 2,500 rpm for 10–15 minutes (centrifuge, micro shaker), and treated with penicillin and streptomycin at 37°C for 30 minutes.

Isolation and Identification

Virus isolation was performed by inoculating 0.1–0.2 mL of the processed suspension into the allantoic cavity of 10-day-old embryonated chicken eggs using

syringes. The eggs were candled (candling lamp), marked, drilled, sealed with nail polish, and incubated at 37°C (incubator) up to five days and monitored daily via candling. Embryonic deaths occurring within the first 24 hours were discarded as non-specific trauma. After 24 hours, eggs with dead embryos or those reaching the end of the incubation period were chilled at 4°C for at least two hours before harvesting. Allantoic fluid was harvested from dead embryos using micropipettes and stored in sterile micro tubes for hemagglutination (HA) and hemagglutination inhibition (HI) tests. The HA and HI tests were performed on U-bottom 96-well micro plates using micropipettes. A 1% red blood cell suspension was prepared from specific pathogen-free chicken blood collected into Ethylenediamine Tetraacetic Acid (EDTA) tubes, washed and centrifuged as per OIE (2021).

Data Analysis

Data were tabulated and analyzed descriptively based on HA/HI results, clinical signs and anatomical pathology changes. Sampling was conducted in Marga and Penebel Districts, with laboratory analysis at the Biomedical and Molecular Biology Laboratory, Faculty of Veterinary Medicine, Udayana University.

RESULTS AND DISCUSSION

Result

To describe the occurrence of Newcastle Disease (ND) and Avian Influenza (AI) H5N1 cases in Marga and Penebel Districts, Tabanan Regency, Bali, sixteen total of 16 head of commercial chickens showing clinical signs of respiratory, digestive and neurological disorders were sampled. The examination results revealed that all samples exhibited clinical signs and anatomical pathology changes, with a predominance of digestive system disorders found in all 16 examined samples. Specifically, 14 samples showed gross lesions in the digestive system, including haemorrhage

in the cecal tonsils, intestines and petechiae in the proventriculus. Nervous system disorders such as torticollis and respiratory symptoms like dyspnoea or lung haemorrhage were observed in nine specific samples (TB1, TB4, TB5, TB6, TB9, TB10, TB11, TB12 and TB15).

Of the sixteen samples tested using the haemagglutination (HA) test, ten samples were positive and six samples were negative. Subsequent serological testing using the haemagglutination inhibition (HI) test with standard ND and AI-H5N1 serum identified that of the ten HA-positive samples, only five were confirmed positive for the target viruses. The HI test results identified four samples (TB5, TB6, TB11, and TB13) as positive for mixed infection of ND and AI-H5N1. Meanwhile, one sample (TB9) was positive for AI-H5N1 infection only. The remaining five HA-positive samples (TB2, TB4, TB7, TB8 and TB10) tested negative in the HI test for both ND and AI-H5N1.

Discussion

Clinical Signs and Pathological Changes. Clinical signs from this study indicating ND and AI-H5N1 in Marga and Penebel Subdistricts involved disorders of the respiratory, digestive and nervous systems (Figure 1). The predominance of digestive system disorders found in all examined samples indicates a tendency for the circulating ND and AI-H5N1 viruses to attack and persist in the digestive system (Mariappan *et al.*, 2018; Tyasasmaya *et al.*, 2016). Meanwhile, the variation in the severity of nervous and respiratory symptoms suggests the possibility of differences in virus virulence or immunity levels among individual chickens (Hewajuli and Dharmayanti, 2011). Based on the study results (Table 1), of the 16 chicken samples experiencing digestive system disorders in the form of greenish-white diarrhoea, six samples tested negative in the HA test, it may be caused by a few reasons, for example disease agents that do not have haemagglutinin, such as *Salmonella gallinarum* and *Escherichia coli*.

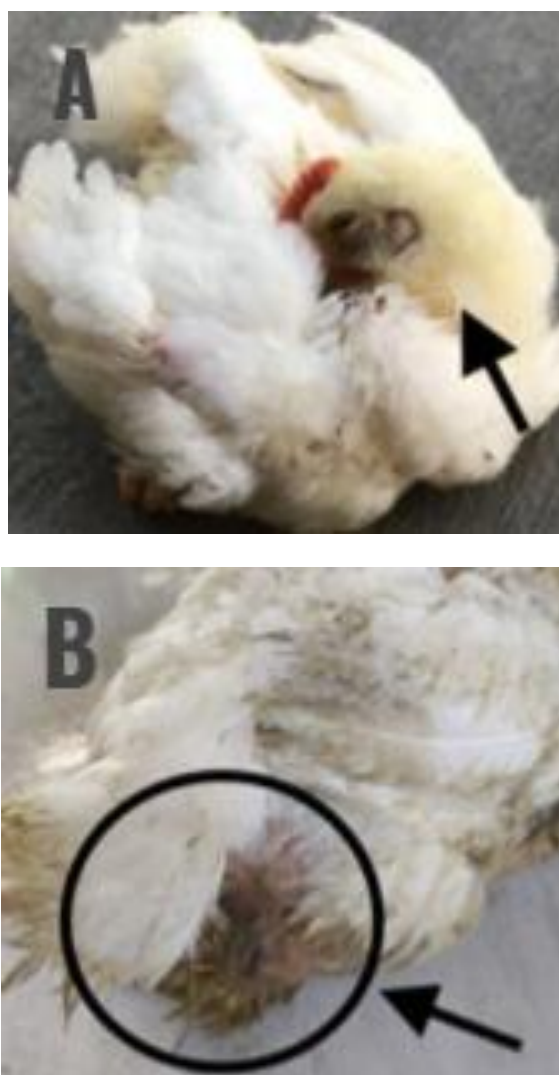


Figure 1. Condition of chicken samples prior to necropsy A) Nervous disorder: torticollis; B) Digestive disorder: presence of fecal stains.

Both bacteria can cause systemic infections with clinical signs of digestive disorders but do not produce haemagglutinin, so they are not detected in the HA test (Kamble *et al.*, 2017). In other case, the cause results from genetic changes. In the case of the AI-H9N2 virus, the K141N point mutation can eliminate HA activity while the virus itself continues to be present and functional (Wen *et al.*, 2024).

Nervous system disorders such as incoordination and torticollis were found in nine samples collected, namely TB1, TB4, TB5, TB6, TB9, TB10, TB11, TB12, and

Table 1. Results of chicken sample testing based on serological examination

Subdistrict	HA Test Positive	HA Test Negative	HI Test (ND Serum)	HI Test (AI-H5N1 Serum)	Both ND and AI-H5N1 Positive
Marga	6	2	2	2	2
Penebel	4	4	2	3	2
Total	10	6	4	5	4

Note : The data represent the serological test result of sampled chickens

Table 2. Summary of findings in chicken samples that tested positive in the HI test

No	Sample Codes	Clinical Findings	Positive HI Test Result
1	TB5	Congestion in the brain, hemorrhage in the intestines, petechiae in the proventriculus, and hemorrhage in the lungs	Positive ND and AI-H5N1
2	TB6	Congestion in the brain, hemorrhage in the intestines, and hemorrhage in the lungs	Positive ND and AI-H5N1
3	TB9	Congestion in the brain	Positive AI-H5N1
4	TB11	Congestion in the brain, hemorrhage in the intestines, and hemorrhage in the lungs	Positive ND and AI-H5N1
5	TB13	Hemorrhage in the intestines	Positive ND and AI-H5N1

Note : TB is the code used to represent samples taken in Tabanan Regency.

TB15. The emergence of disorders in the nervous system that is not detected in HA test may be caused by the viral load in the brain tissue did not reach the required threshold since the haemagglutination (HA) assay necessitates 10^4 to 10^5 viral particles per mL, the results remained negative despite the presence (false negative) of the virus (Howley and Knipe, 2020).

Respiratory disorders in chickens such as rales, coughing, dyspnoea, and oedema of the comb found in samples TB2, TB4, TB5, TB6, TB7, TB10, TB11, TB12, and TB16. The negative HA test in some samples might happen because haemagglutinin protein is highly sensitive to heat, extreme pH changes, or bacterial contamination within the sample. If the cold chain is compromised during transport to the laboratory, the HA protein can become denatured. While the virus may still be detectable via polymerase chain reaction (PCR) as the genetic material remains present, but it cannot be detected by

HA test (Swayne, 2020).

Anatomical pathology changes observed in broiler chickens included haemorrhage in the lungs, congestion in the brain, petechiae in the proventriculus, and haemorrhage in the cecal tonsils and intestines (Figure 2). This is in line with Zhang *et al.* (2023) regarding the anatomical pathology changes in ND, involving organs of the digestive, nervous and respiratory systems.

The anatomical pathology findings showed that lesions in the digestive system were the most frequently found. Of all the samples examined, 14 samples experienced changes in the digestive system. This finding is consistent with the report of Kabiraj *et al.* (2020) on ND anatomical pathology lesions, including haemorrhage in the cecal tonsils, intestines and petechiae in the proventriculus, in accordance with field findings in ND cases. Lesions in the nervous system were also found in organ samples TB1, TB4, TB5, TB6, TB9, TB10, TB11, TB12, a

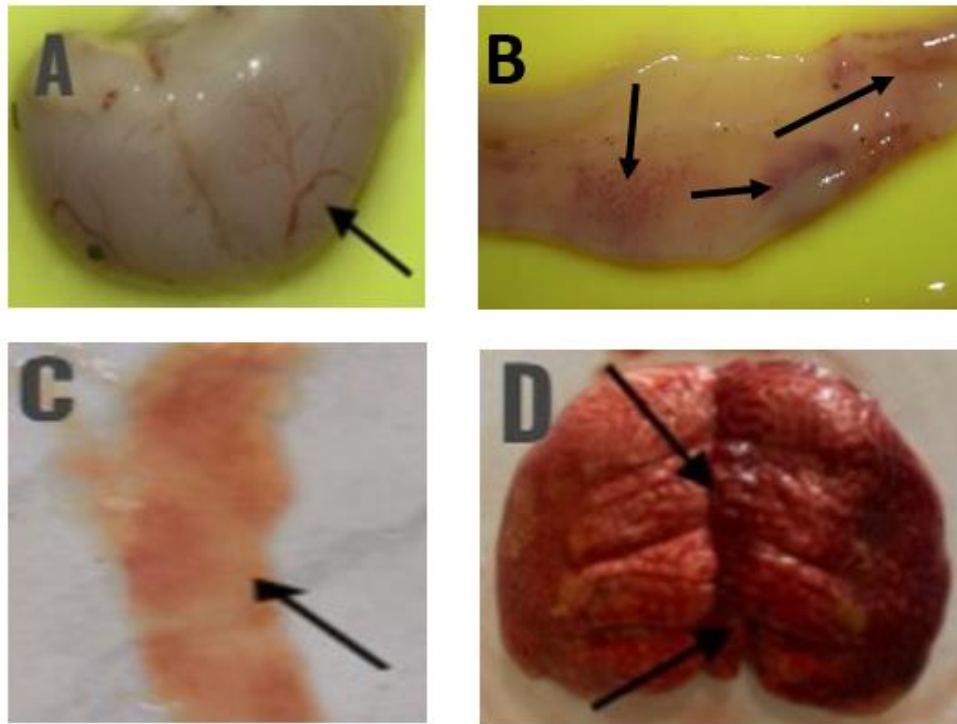


Figure 2. Anatomical pathology findings A) Congestion in the brain; B) Hemorrhage in the intestine; C) Hemorrhage in the cecal tonsils; D) Hemorrhage in the lungs.

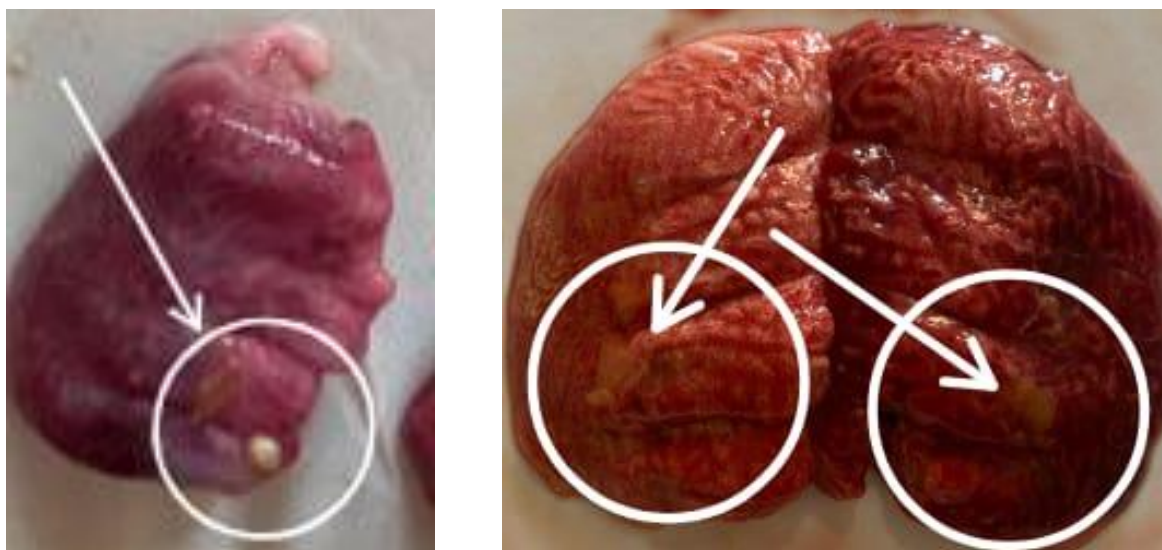


Figure 3. Anatomical pathology findings. Presence of fibrinous exudate in the lungs.



Figure 4. Haemagglutination (HA) test result

Notes: negative control (yellow border); titre 2^6 (red border); positive control (black border)

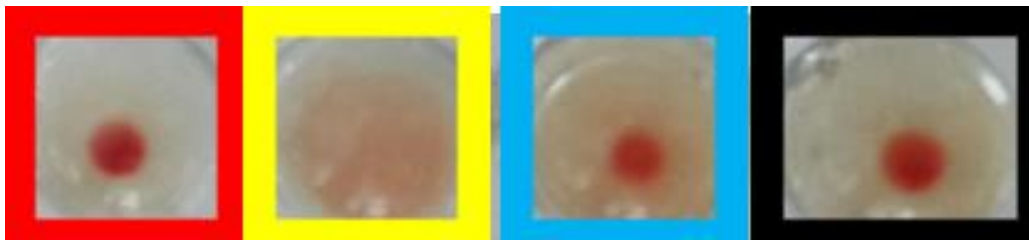


Figure 5. Hemagglutination Inhibition (HI) test results for ND and AI

Notes: red blood cell control (red border); antigen control (yellow border); AI positive haemagglutination inhibition observed (blue border); ND positive haemagglutination inhibition observed

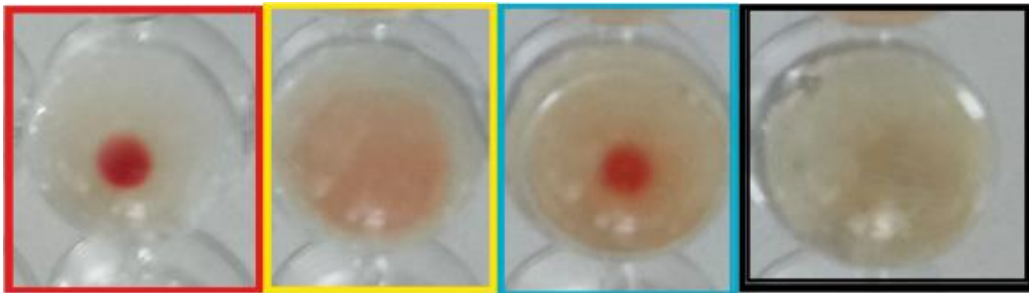


Figure 5. Hemagglutination Inhibition (HI) test results for ND and AI

Notes: red blood cell control (red border); antigen control (yellow border); AI positive haemagglutination inhibition observed (blue border); ND negative haemagglutination inhibition observed

TB15, indicating the presence of disease agents that cause anatomical pathology changes. As found by Nishiura *et al.* (2020), Avian Leucosis' Virus from the *Alpharetrovirus* genus, which does not have haemagglutinin, causes lesions in the nervous system, especially in the brain such as encephalitis and glioma.

Lesions found in the respiratory system organs in samples TB2, TB4, TB5, TB6, TB7, TB10, TB11, TB12, and TB16 showed that the lungs experienced haemorrhage indicating changes leading to ND and AI-H5N1. However, the HA test showed that there were samples that experienced changes but did not show positive results in the HA test.

The anatomical pathology examination of lung tissue (Figure 3) revealed the deposition of fibrin exudates in samples TB8 and TB11. This finding can be associated with infection by disease such as colisepticemia caused by *E. coli*, a bacterium that does not produce haemagglutinin (Panth, 2019).

Analysis Based on HA/HI Tests

Clinical and anatomical pathology examinations revealed the presence of ND and AI-H5N1. To confirm the diagnosis, HA/HI tests were conducted as stated in the study by Mao *et al.* (2022), and these tests were used as confirmatory methods to detect the presence of viruses in samples. The test results showed a correlation between clinical signs, pathological changes and the presence of ND and AI-H5N1 viruses confirmed serologically, thereby strengthening the diagnosis of ND cases in the tested samples. In this study. The HA and HI test results is presented in Figure 4 and Figure 5. Meanwhile Choi *et al.* (2013) explained that the HA test works by detecting viral antigens based on their ability to bind and agglutinate erythrocytes and the HI test functions to identify specific antibodies against ND and AI-H5N1 viruses using standard sera. In this study, only a rapid HI test was performed using standard ND and AI-H5N1 sera from

Pusvetma Surabaya without calculating antibody titres.

The HI test results using ND and AI-H5N1 sera showed that several samples, namely TB5, TB6, TB11, and TB13, tested positive against both types of sera. This finding indicates mixed infections, where chickens infected with ND were also simultaneously infected with AI-H5N1 virus as shown in Figure 5. This condition reinforces the suspicion that, in some field cases, ND virus infection does not occur alone but can coincide with AI-H5N1 virus infection (Gowthaman *et al.*, 2019). Mixed infections between ND and AI-H5N1 can occur due to several factors, such as simultaneous exposure in the environment or infection by one virus like NDV, which can suppress the chicken's immune system, thereby facilitating infection by other viruses such as AI-H5N1 (Hassan *et al.*, 2016; Musa *et al.*, 2020).

Among the samples that tested positive in the HI test using ND and AI-H5N1 sera, sample TB9 (Figure 6) showed a positive result for infection with AI-H5N1 only. Not all chickens are exposed to equal amounts of both viruses, if exposure to the ND virus is lower or does not occur in certain samples, only AI-H5N1 will be detected (Ripa *et al.*, 2021).

Of the ten samples that tested positive in the HA test, five (TB5, TB6, TB9, TB11 and TB13) gave positive results in the HI test, while the other five samples (TB2, TB4, TB7, TB8 and TB10) showed negative results. Negative HI results in HA-positive samples indicate that the causative agents possess haemagglutinin protein but are not caused by ND or AI-H5N1 viruses. Other diseases that also have haemagglutinating ability and can cause systemic symptoms in the nervous, digestive and respiratory systems besides ND and AI-H5N1 include Infectious Coryza (snot disease) in chickens caused by *Avibacterium paragallinarum*. A study by Potehin *et al.* (2019) showed that *A. paragallinarum* isolates exhibited haemagglutination activity when tested. Other AI-mivirus subtypes such as H3N3, H9N2 and

H7N7 also have haemagglutinin proteins and can cause systemic infections in poultry, including respiratory and digestive symptoms. Some of these subtypes are low pathogenic in poultry and have become increasingly adaptive, thus going undetected in HI tests using AI-H5N1 serum (Wan *et al.*, 2023).

Relevance of Field ND Virus to the Standard Serum

The relevance between field strains of ND and AI-H5N1 viruses and the standard sera from Pusvetma Surabaya used in serological tests, such as the HI test, is a key determinant of diagnostic and surveillance accuracy (Mao *et al.*, 2022). The haemagglutinin (HN protein) of ND and AI-H5N1 viruses serves as a key antigen that interacts with specific antibodies in the serum. The structural compatibility of the HN protein epitope with the standard serum antibodies determines the success of the haemagglutination inhibition reaction (Jin *et al.*, 2021). If mutations occur in the HN protein epitope, the standard serum may fail to recognize the field virus antigen, potentially resulting in false-negative outcomes that can interfere with the early detection of ND infection (Moustapha *et al.*, 2023). As in the case of TB4, the observed clinical signs and anatomical pathology changes were highly consistent with ND infection, but the HI test results using standard ND and AI-H5N1 sera were negative.

Another possibility of a negative HI result is that this study only used a rapid HI test, making it less sensitive, resulting in a negative HI result (false negative). It is important to note that this is not considered to be uninfected by the ND or AI virus because the ND virus in Bali has mutated with the discovery of ND Genotype VIIi (Kencana *et al.*, 2025) which was previously ND Genotype VIIh (Adi *et al.*, 2010), although the ND virus mutation is not as fast as the AI virus. The AI-H5N1 virus in Bali has also mutated with the report of the AI-H5N2 clade 2.2 virus in chickens from

Penebel, Bali (Kencana *et al.*, 2020). Therefore, further testing with PCR and sequencing is needed to obtain more accurate test results.

The effectiveness of the HI test as a diagnostic method also depends on the antigenic match between the field virus strain and the standard serum (Choi *et al.*, 2013). Antigenic mismatch, for example due to mutations in the HN gene that alter epitope structure, can reduce the sensitivity of the HI test. This poses a risk of inaccurate test results, such as failure to detect the presence of antibodies that actually exist in the sample (Lu *et al.*, 2022).

The Correlation Between Clinical, Pathology Signs and Serological Tests

Based on the results of the study, the correlation between clinical signs, anatomical pathology changes and serological test results appeared quite evident, although not entirely consistent. In Marga and Penebel Subdistricts, chicken samples exhibited clinical signs in the form of digestive, respiratory and nervous disorders, yet only 10 out of 16 samples were confirmed positive through the HA test, and five out of 16 samples tested positive through the HI test using ND and AI-H5N1 sera. This indicates that although clinical signs and macroscopic lesions in organs were visible, ND and AI-H5N1 virus infections still require laboratory confirmation through serological tests to ensure an accurate diagnosis, considering factors that may affect the detection of viruses or antibodies (Pranatha *et al.*, 2018).

CONCLUSION

It can be concluded that chickens suspected of being infected with Newcastle Disease (ND) virus and Avian Influenza-H5N1 (AI-H5N1) virus showed clinical signs in the form of disorders of the digestive system, respiratory system, and nervous system. The most frequently observed anatomical pathology changes were haemorrhage in the lungs, intestines, cecal tonsils, and petechiae in the proventriculus, as well as con-

gestion in the brain.

These findings confirm that Virus isolation using embryonated chicken eggs and HA/HI tests are effective in identifying ND and AI-H5N1 infections in field cases. However, some cases with clinical signs and anatomical pathology characteristic of ND and AI-H5N1 were not serologically confirmed.

SUGGESTION

In conducting this study, the researcher faced limitations in terms of sample size, funding, media and time. Therefore, to support better research outcomes in the future, the researcher hopes that subsequent studies can address the gaps in this study and further improve upon it.

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REFERENCES

- Adi A AAM, Astawa NM, Putra KSA, Hayashi Y, and Matsumoto Y. 2010.. Isolation and characterization of a pathogenic Newcastle disease virus from a natural case in Indonesia. *Journal of Veterinary Medical Sciences*, 72(3): 313-319. DOI: <https://www.doi.org/10.1292/jvms.09-0303>
- Ayuti SR, Khairullah AR, Lamid M, Al-Arif MA, Warsito SH, Silaen OSM, Aryaloka S. 2024. Avian influenza in birds: Insights from a comprehensive review. *Veterinary World* 17(11): 2544–2555. <https://doi.org/10.14202/vetworld.2024.2544-2555>
- Choi KS, Kye SJ, Jeon WJ, Park MJ, Kim S, Seul HJ, Kwon JH. 2013. Preparation and diagnostic utility of a hemagglutination inhibition test antigen

- derived from the baculovirus-expressed hemagglutinin neuraminidase protein gene of Newcastle disease virus. *Journal of Veterinary Science* 14(3): 291–297. <https://doi.org/10.4142/jvs.2013.14.3.291>
- Emilia A, Setiyaningsih S, Soejoedono RD. 2015. Isolation and Biological –Characterization of Newcastle Disease Virus. *Jurnal Kedokteran Hewan* 9(1): 47–51. <https://doi.org/10.21157/j.ked.hewan.v9i1.278>
- Gowthaman V, Singh SD, Dhama K, Ramakrishnan MA, Malik YPS, Gopala KMTR, Munir M. 2019. Co-infection of Newcastle disease virus genotype XIII with low pathogenic avian influenza exacerbates clinical outcome of Newcastle disease in vaccinated layer poultry flocks. *Virus Disease* 30(3): 441–452. <https://doi.org/10.1007/s13337-019-00533-6>
- Hassan KE, Shany SAS, Ali A, Dahshan AHM, El-Sawah AA, El-Kady MF. 2016. Prevalence of avian respiratory viruses in broiler flocks in Egypt. *Poultry Science* 95(6): 1271–1280. <https://doi.org/10.3382/ps/pew068>
- Hewajuli DA, Dharmayanti NLPI. 2011. Pathogenicity of Newcastle Disease Virus in Chickens. *Wartazoa* 21(2): 72–80.
- Howley PM, Knipe DM. (Eds.). 2020. *Fields Virology: Emerging Viruses* 7th ed. Vol. 1. Philadelphia. Wolters Kluwer Health.
- Jin Z, Wei Q, Bi Y, Li Y, Huo N, Mou S, Xiao S. 2021. Identification of a potential neutralizing linear epitope of hemagglutinin-neuraminidase in Newcastle disease virus. *Virology Journal* 18(1): 1–11. <https://doi.org/10.1186/s12985-020-01483-y>
- Kabiraj CK, Mumu TT, Chowdhury EH, Isam MR, Nooruzzaman M. 2020. Sequential pathology of a genotype XIII Newcastle disease virus from Bangladesh in chickens on experimental infection. *Pathogens* 9(7): 1–14. <https://doi.org/10.3390/pathogens9070539>
- Kamble NM, Hyoung KJ, Lee JH. 2017. Intracellular delivery of HA1 subunit antigen through attenuated *Salmonella gallinarum* act as a bivalent vaccine against fowl typhoid and low pathogenic H5N3 virus. *Veterinary Research* 48(1): 1–12. <https://doi.org/10.1186/s13567-017-0446-1>
- Kencana GAY, Kardena IM, Mahardika IG NK. 2012. Confirmation of Newcastle Disease Diagnosis in Free-Range Chickens in Bali Using RT-PCR Technique. *Jurnal Ilmiah Kedokteran Hewan* 6(1): 50–53. <https://doi.org/10.21157/j.ked.hewan.v6i1.349>
- Kencana GAY, Suartha IN, Kardena IM, Agustina KK. 2021. Seroprevalence and detection of H5N1 avian influenza virus in local chickens in Tabanan Regency, Bali, Indonesia. *Acta Veterinaria Indonesiana* 9(3): 223–229. <https://doi.org/10.29244/avi.9.3.223-229>
- Kencana GAY, Suartha IN, Kardena IM and Nurhandayani A, 2020. Isolation and characterization of avian influenza virus H5N1 subtype field isolate from bali for vaccine. *Jurnal Veteriner* 21(4): 530-538. <https://doi.org/10.19087/jveteriner.2020.21.4>
- Kencana GAY, Astawa INM, Kendran AAS, Pratomo AA. 2025. Assessment of Active LaSota and Inactivated Newcastle Vaccines and Their Nucleotide Sequence Homology on the Virulence of Wild-type Newcastle Disease Genotype VIIi Virus. *World's Veterinary Journal* 15(3): 678-687. DOI: <https://dx.doi.org/10.54203/scil.2025.wvj68>
- Lu X, Liu X, Song Q, Wang X, Hu S, Liu X. 2022. Amino Acid Mutations in Hemagglutinin-Neuraminidase Enhance the Virulence and Pathogenicity of the Genotype III Newcastle Disease Vaccine Strain After Intravenous Inoculation. *Frontiers in Veterinary Science* 9: 1–13. <https://doi.org/10.3389/fvets.2022.888888>

- [fvets.2022.890657](https://doi.org/10.1098/rstb.2018.0257)
- Lycett, S. J., Duchatel, F., and Digard, P. 2019. A brief history of bird flu. *Philosophical Transactions of the Royal Society B: Biological Sciences* 374(1775): 1–3. <https://doi.org/10.1098/rstb.2018.0257>
- Mao Q, Ma S, Schrickel PL, Zhao P, Wang J, Zhang Y, Wang C. 2022. Review detection \of Newcastle disease virus. *Frontiers in Veterinary Science* 9(2). <https://doi.org/10.3389/fvets.2022.936251>
- Mariappan AK, Munusamy P, Kumar D, La theef SK, Singh SD, Singh R, Dhama K. 2018. Pathological and molecular investigation of velogenic viscerotropic Newcastle disease outbreak in a vaccinated chicken flocks. *Virus Disease* 29(2): 180–191. <https://doi.org/10.1007/s13337-018-0445-5>
- Moustapha A, Talaki E, Akourki A, Ousseini M. 2023. Newcastle Disease Virus in Poultry: Current Status and Control Prospects. *World's Veterinary Journal* 13(2): 240–249. <https://doi.org/10.54203/scil.2023.wvj26>
- Musa WI, Sa'idu L, Bello M, Abdu PA. 2020. Co-infections of domestic and wild birds with avian influenza and Newcastle disease viruses: implications for control and genetic mutations. *Veterinary Research Communications* 44(3–4): 159–166. <https://doi.org/10.1007/s11259-020-09783-y>
- Nishiura H, Kubota I, Kondo Y, Kachi M, Hatai H, Sasaki J, Ochiai K. 2020. Neuropathogenicity of newly isolated avian leukosis viruses from chickens with osteopetrosis and mesenchymal neoplasms. *Avian Pathology* 49(5): 440–447. <https://doi.org/10.1080/03079457.2020.175762>
- OIE [Office International des Epizooties]. 2012. *Manual of Diagnostic Test and Vaccines for Terrestrial Animal Chapter 2.3.4. Avian Influenza pp.1-21; Chapter 2.3.14. Newcastle Disease Pp. 1-9 Paniago M. 2007. Paris. OIE.*
- OIE [Office International des Epizooties]. 2021. *Newcastle Disease OIE Terrestrial Manual 2021. Paris. OIE.* https://www.woah.org/fileadmin/Home/eng/Health_standards/tahm/3.03.04_AI.pdf
- Panth Y. 2019. Colibacillosis in poultry: A Review. *Journal of Agriculture and Natural Resources* 2(1): 301-311.
- Potehin AV, Yevgrafova VA, Andreychuk DB. 2019. Hemagglutination and antigenic properties of *Avibacterium paragallinarum* isolates recovered in the Russian Federation and Republic of Belarus. *Veterinary Science Today* 4: 31-38.
- Pranatha WD, Irhas R, Arhiono HNP, Widya santi NWH, Kardenia IM. 2018. Case Report of Newcastle Disease and Avian Influenza in Native Chicken. *Indonesia Medicus Veterinus* 7(5): 498–507. <https://doi.org/10.19087/imv.2018.7.5.498>
- Ripa RN, Sealy JE, Raghwanj J, Das T, Barua H, Masuduzzaman M, Biswas PK. 2021. Molecular epidemiology and pathogenicity of H5N1 and H9N2 avian influenza viruses in clinically affected chickens on farms in Bangladesh. *Emerging Microbes and Infections* 10(1): 2223–2234. <https://doi.org/10.1080/221751.2021.2004865>
- Swayne DE, Suarez DL. 2020. Avian influenza. In Swayne DE (Ed.), *Diseases of poultry* 14th ed., Hoboken, New Jersey. Wiley-Blackwell. Pp.210–256.
- Tyasasmaya T, Wuryastuty H, Wasito H, Sievert, K. 2016. Avian Influenza Virus H5N1 Remained Exist in Gastrointestinal Tracts of House Flies 24 Hours Post-infection. *Jurnal Veteriner* 17(2): 205–210. <https://doi.org/10.19087/jveteriner.2016.17.2.205>
- Wan Z, Zhao Z, Sang J, Jiang W, Chen J, Tang T Ye J. 2023. Amino Acid Variation at Hemagglutinin Position 193 Impacts the Properties of H9N2 Avian Influenza Virus. *Journal of Virology* 97(2). <https://doi.org/10.1128/jvi.01379-22>
- Wen F, Yan Z, Chen G, Chen Y, Wang N, Li Z, Huang S. 2024. Recent H9N2 avian influenza virus lost hemagglutination activity due to a K141N substitution in hemagglutinin. *Journal of Virology* 98(4): e00248-24.
- Zhang D, Ding Z, Xu X. 2023. Pathologic Mechanisms of the Newcastle Disease Virus. *Viruses* 15(4). <https://doi.org/10.3390/v15040864>