Chapter

The Thrift of Avian Influenza in Indonesia

Khrisdiana Putri, Sitarina Widyarini, Sugiyono and Widya Asmara

Abstract

The circulating H5N1 Highly Pathogenic Avian Influenza in chicken has created devastating problems in Indonesia since 2003. Although human cases of Avian Influenza could be exceptionally reduced, however, it remains unsettled in poultry. Phylogenetic analysis of H5N1 virus (2003–2011) revealed the introduction of a single ancestral of 2.1 HA clade before 2003. The enzootic clade subsequently evolved into fourth order with predominantly 2.1.3.2. Pathological lesions showed cyanotic wattle, torticollis and haemorrhage in chicken feet and multi-internal organs. However, the introduction of vaccination and stringent biosecurity resulted in milder manifestations compared to classical lesions. In 2012, unusual high mortality in duck farms revealed the introduction of exotic clade 2.3.2.1. Despite the inefficient transmission of avian virus to humans and experimental receptor binding of 2.3.2.1 virus that showed avian preference, substitution of N158D and E190D in HA gene indicates possible threat to humans. In the same year, the Government of Indonesia announced the introduction of H9N2. Furthermore, a recent publication (2018) has reported new reassortant between HPAIV H5N1 and LPAIV H3N8 with resulting virulence attenuation in chicken.

Keywords: chicken, Indonesia, antigenic thrift, pathological lesion

1. Introduction

Avian Influenza (AI) is influenza A virus of avian origin, which may cause disease in domestic and wild birds and in some cases can infect mammalian species, including humans. The highly pathogenic variant (HPAI) has spread to more than 60 countries in Africa, Asia, Australia, Europe and North and South America only within decades. The disease has continuously involved in detrimental impact to poultry farms despite global efforts towards control and eradication. The Indonesian lineage has attracted human health community for its zoonotic attribute by demonstrating the capacity for causing three family cluster cases (West Java, Banten and North Sumatera) with one of them being the largest case in human AI history [1–3]. However, surveillance of H5N1 antibody in poultry farmers from human H5N1 outbreak areas was reported and not detected [4].

Molecular identification on samples obtained during surveillance for H5N1 virus in municipal of Muntilan, Center Java, conducted by Regional Influenza Working Group, after suspected human H5N1 infection announced in 2005, were able to identify H5N1 virus in pet animals and fish pond in the housing areas. However, virus

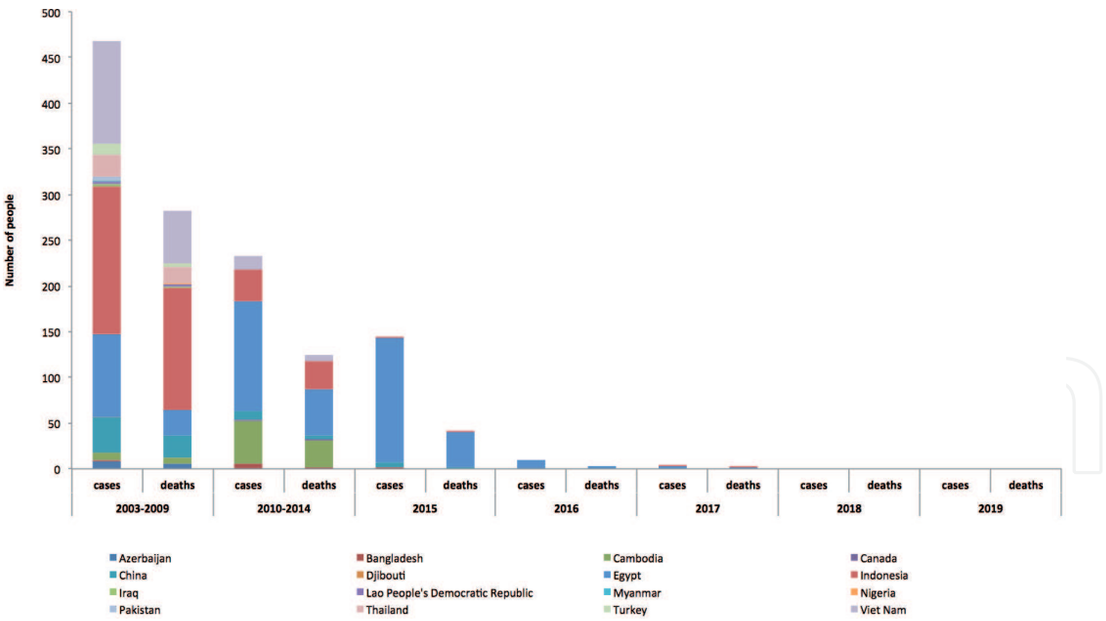

Figure 1.
Number of human Avian Influenza A (H5N1) cases by reporting country and month of onset (Taken from the World Health Organisation. Influenza at human-animal interface. Summary and assessment as of 1 May 2015 https://www.who.int/influenza/human_animal_interface/Influenza_Summary_IRA_HA_interface_1_May_2015.pdf).

sequences are not available. The number of human deaths in Indonesia were outgrowing to 150 by 2011 (**Figure 1**) [2, 5–7] with 46% reported to have direct contact with infected poultry [7]. Although, to date the virus demonstrated inefficient person-to-person transmission, ongoing outbreaks in poultry pose warning to possibly establish human reassortant Avian Influenza virus [8]. New outbreaks of H5N1 in 2014 in Cambodia, China, India, Korea, Lybia, Russia and Vietnam have shown high adaptability in a heterogeneous ecosystem, requiring urgent need for reliable surveillance tool to improved strategies to control and eradicate this enzootic disease.

2. Host specificity of Asian lineage virus

Part of the HA protein that binds to the host receptor [called the receptor binding site (RBS)] has a unique amino acid arrangement which contributes to viral specificity to the host [9].

Infection occurs when the viral ligand binds to a glycoprotein or glycolipid receptors on the cell surface possessing sialylgalactose terminal group [Neu5Ac (α2-3) Gal] or [Neu5Ac (α2-6) Gal]. Influenza virus of 226Gln and 228Gly avian origin prefers to bind to [Neu5Ac (α2-3) Gal], while influenza virus of 226Leu and 228Ser human origin binds specifically to [Neu5Ac (α2-6) Gal] [9, 10]. The fact the epithelial cells of human respiratory tract mainly contain [Neu5Ac (α2-6) Gal], while the majority in chicken is [Neu5Ac (α2-3) Gal], has provided an explanation the avian origin virus cannot readily infect humans. The shift in host specificity is possible due to the changes in amino acids in RBS through genetic mutations. Experimentally substituting an amino acid of Ser228Gly in addition to Leu226Gln of human origin virus has supported viral replication in duck intestines [11]. Although, solely mutation event of single amino acid in RBS was adequately altering binding specificity to the receptor [12, 13]. Amino acid substitution Ser227Asn in highly pathogenic avian influenza virus (HPAIV) H5N1 of Asia strain decreases its affinity for the receptor [Neu5Ac (α2-3) Gal] and gives the virus ability to bind to [Neu5Ac (α2-6) Gal] moderately. This indicates that mutations in RBS are capable to induce cross-species transmission without genetic reassortment [14].

A genetic rearrangement between influenza viruses of avian origin and influenza viruses from mammals has the potential to emerge new pandemic influenza virus strains in humans. Classical genetic reassortment model has settled pigs as mixing vessel to both viruses. The basis of the model is the specificity of the influenza virus strain to the host cell surface receptors [15, 16].

The emergence of four influenza pandemics, 1918 (H1N1), 1957 (H2N2), 1968 (H3N2), and 1977 (H1N1) was not due to genetic reassortment in pigs. The specificities of the receptors in haemagglutinin gene of 1918 virus vary between strains. Isolates A/South Caroline/1/18 tend to bind to [Neu5Ac (α2-6) Gal] receptors, while isolates A/New York/1/18 have the ability to bind both [Neu5Ac (α2-6) Gal] and [Neu5Ac (α2-3) Gal] receptors. Compared to the H1 virus from avian origin in general, isolates A/New York/1/18 differ only in amino acid 190. The viral HA mutation in this position from Asp to Glu decreases the ability of the virus to bind to the [Neu5Ac (α2-6) Gal] receptor and increases preference to the [Neu5Ac (α2-3) Gal] (avian receptor) [13]. The avian influenza virus that caused the outbreak in Asia in 2003–2004 did not show such characteristics. Some viruses isolated from Vietnam, Thailand, Hong Kong and Indonesia, both from human and avian, show similarities in amino acid sequences in the RBS area and have a preference for binding to [Neu5Ac (α2-3) Gal] (avian receptors) [12, 17–19].

3. Indonesia situation

Highly Pathogenic Avian Influenza (HPAI) has been a major problem for poultry industry in Indonesia till today. Since first announced in 2003–2004 (**Figure 2**), H5N1 outbreak was rapidly spread to most provinces, before abated by the end of 2007, after causing death to more than 16 million poultry [2, 5, 7]. In April 2011, a new outbreak was reported from Gorontalo, leaving only one province free of disease [20].

Phylogenetic analysis of Indonesian 2.1. clade virus indicated direct precursor-descendant link to viruses of genotype Z, isolated from Hunan province, China in 2002, presumably as single introduction. However, the spread and transmission from Hunan to Indonesia remained unclear [21, 22].

Up to the year 2008, all Indonesian H5N1 viruses have been classified into clade 2.1, with three virus sublineages: 2.1.1, 2.1.2 and 2.1.3. The viruses within clade 2.1.1 were mainly isolated from HPAI-infected poultry during the outbreaks between

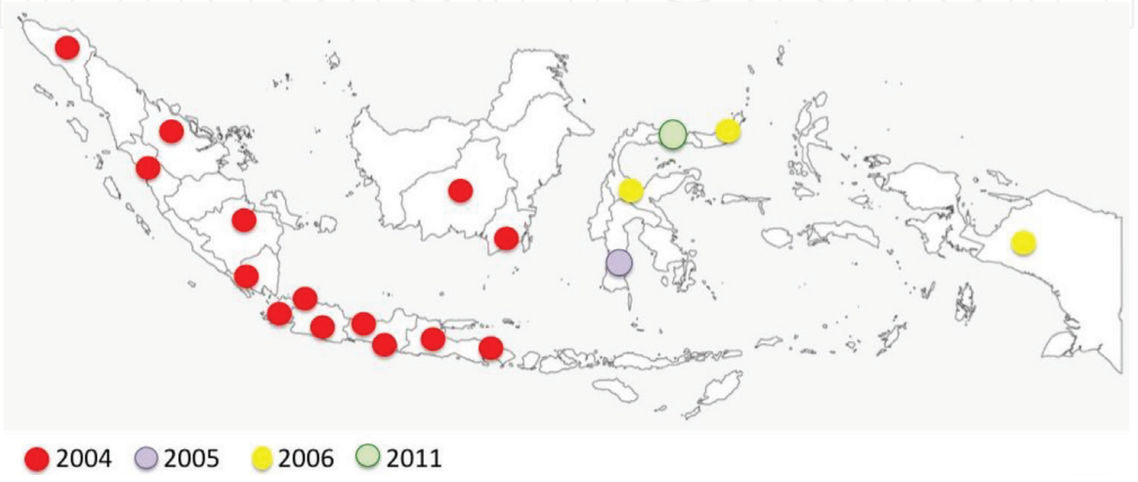

Figure 2.
H5N1 Avian Influenza poultry epidemic in Indonesia. (Map by Free Vector Maps. https://freevectormaps.com/indonesia/ID-EPS-01-0003).

2003 and 2005. The clade 2.1.2 viruses were isolated from avian- and human-derived predominantly from Sumatra between 2004 and 2007, while clade 2.1.3 viruses discovered in 2004, were isolated either from birds or from humans. Interestingly, when clade 2.1.3 viruses have begun to predominate, the numbers of clade 2.1.1 and 2.1.2 isolates were subsequently declined. Although 2.1.3 viruses have spread and become endemic in many provinces in Indonesia, a new sublineage virus has emerged since 2004. In September 2012, several duck farms from Central Java have reported high mortality of AIV H5 subtype. Interestingly, the HA genes of the duck isolates were not related to long-established Indonesian clade 2.1 isolates but closely resembled clade 2.3.2.1 viruses, which recently were found in Vietnam, China and Hong Kong [23].

Bali Island has reported only one human death because of Avian Influenza until 2017, although Bali is speculated as an ideal environment for influenza re-assortment: world-renowned tourism destination, suckling pigs, and fighting cocks tradition. Circulating A(H5N1) viruses obtained during surveillance of A(H5N1) viruses in Bali between 2009 and 2011 concluded clade 2.1 [24, 25]. Although incident of human death has occurred in Bali, the HA gene analysis at 226Q and 228G of chicken isolates yet showed binding preference to avian host. However, a single mutation finding at S137A has shown the potential of recognizing human receptor. Although evolution analysis of obtained isolates from Bali (A/Ck/Klungkung/T/2009 and A/Ck/Bali/Y/2009) is unable to determine due to lack of HA gene sequences of Indonesian isolate available in GenBank, phylogenetic analysis has clustered these isolates with the only Indonesian domestic cat virus (**Figure 3**). Consistent with the outbreak in Thailand, the HA gene of pigeon, chicken, tiger, and human isolates were closely related [26]. The potency of pigs as a mixing vessel for avian virus to adapt in human host is also unable to analyze due to the lack of available sequences in GenBank. However, the phylogenetic analysis of swine virus from Bali showed a close relation to other pig and chicken viruses within the corresponding year [27].

Surveillance of A(H5N1) viruses in live bird markets (LBM) during 2012-2013 indicated that most viruses were HPAIV (H5N1), which were related to other clade 2.1.3.2a viruses. The surveillance also detected LPAIV A (H3N8) A/environment/West Java/KRW54/2012, which forms outlier with other LPAI H3 of Eurasian lineage. The A (H3N8) also demonstrated 90% nucleotide identical to A/Duck/Siberia/100/2001. Importantly, genetic reassortment among AIV isolates is occurred by contribution of internal and NA gene segments of LPAIV virus into HPAIV (H5N1) clade 2.1.3.2a virus. Three reassortant viruses (A/Muscovy Duck/East Java/SB29/2012, A/Muscovy Duck/East Java/LM47/ 2012 and A/Ck/East Java/BP21/2012) possessed PB2, PB1 and NS genes of LPAI virus, while the surface glycoproteins (HA and NA) and other internal genes (PA, NP and M) were contribution of HPAI A(H5N1) virus lineage. The experimental data of the reassortant HPAI A(H5N1) viruses showed slight attenuation possibly due to acquisition of LPAI internal genes to HPAI virus [8]. In 2017, the government of Indonesia has officially announced the introduction of enzootic H9N2 subtype; however, it is still poorly documented. The introduction of LPAIV A(H9N2) may possess new hidden endemic zoonotic threat. Chinese Centre for Diseases Control and Prevention has highlighted the role of H9N2 as "incubators" to facilitate new zoonotic human avian strain [28].

Since 2004, the Indonesian Government have been applying vaccination in poultry to control AIV H5N1 and simultaneously intensify biosecurity in poultry farm, conducting active diseases surveillance, application of stamping-out policy limited to endemic area and extensive to newly infected area, and improving public awareness of the disease [29, 30]. Although vaccine can be used as a prevention tool, it does not provide full protection or "sterilising immunity" [31]. Vaccine application for Avian Influenza in the field is recommended to allow to serologically differentiate vaccinated birds from infected (DIVA) [32–34]. Proposed strategy for DIVA by the use of sentinel

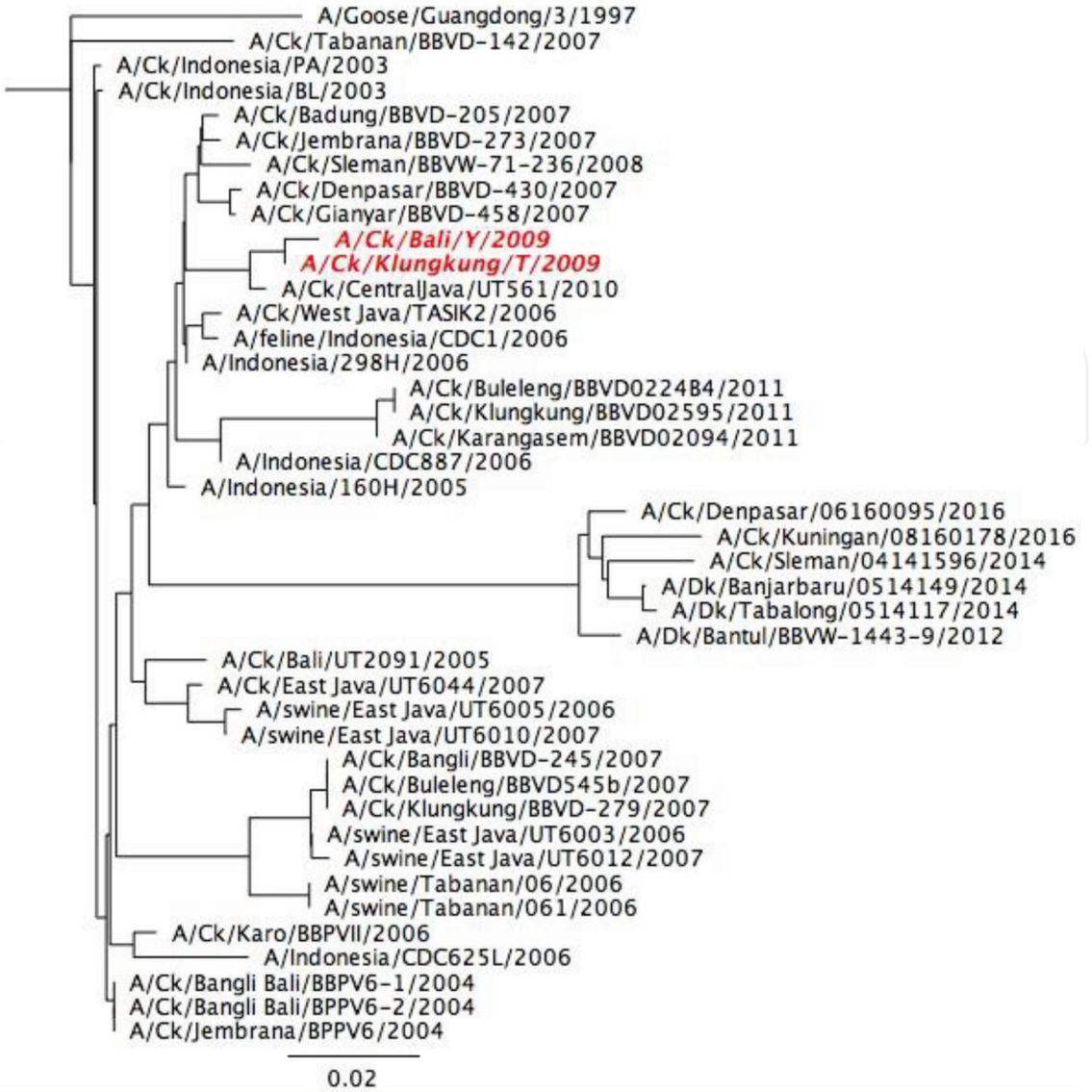

Figure 3.
Phylogenetic tree analysis of A/Ck/Klungkung/T/2009 and A/Ck/Bali/Y/2009 A (H5N1) compared to other sequences of poultry, human, swine and human Indonesia available in GenBank. A/Goose/Guangdong/1997 was served as outgroup in rooted neighbour-joining calculation. The scale indicated 0.02 nt substitution per site. The analysis was performed using Geneious R.10 (Biomatters, Ltd).

chickens has been conducted in West Java [30, 35]. However, as possible, new infections in the flock may originate from these sentinel naive birds, which may acquire infection prior to being placed; this DIVA strategy has not received widespread acceptance in Indonesia. Several alternative strategies using viral protein for marker in chickens have been developed, that is, NS1 [36, 37], M2e [38, 39] and HA2 [40, 41].

4. Pathological features

The pathological features of Avian Influenza infection in poultry since the first outbreak in Indonesia have undergone slight changes over time. The pathological changes are currently showing milder description compared to classical discovery in the middle of 2003. Avian Influenza viruses in poultry were reported to produce asymptomatic to mild upper respiratory infections, egg production loss to rapid fatal systemic disease [42].

Pathogenicity attributes of AI virus were categorised as low pathogenic avian influenza virus (LPAIV) and highly pathogenic avian influenza viruses (HPAIV) [43, 44].

The low pathogenic variant (LPAI) in poultry describes signs of respiratory diseases [43, 45], while high pathogenic variant (HPAI) demonstrates severe systemic signs with necrotic and inflammatory lesions of skin, viscera and brain [46–48], although mortality may occur in the absence of clinical signs [42]. The degree of clinical manifestations and recovery rate of the birds are notably age-related. Older birds generally recover within a week, since the onset of clinical signs. Conversely, younger birds are suffering from severe respiratory symptoms as of reflecting in high mortality rates (40–97%). Furthermore, co-infection of other secondary pathogens also contribute to high mortality [45]. Low-pathogenic infection is typically demonstrating low mortality (<5%) accompanied by high morbidity (>50%) [44, 45], contrarily, infection by HPAI virus results in 100% mortality of susceptible poultry species [43, 48].

Low pathogenic variant AI demonstrates clinically mild to severe respiratory signs, i.e., coughing, sneezing, swollen infraorbital, excessive ocular and nasal discharge [43, 44]. Infected birds, in general show lethargy, mild weight loss, neurological signs, occasional diarrhoea and sudden drop in eggs production from 30 to 80% during acute phase [43–45, 49]. In humans, a high viral load in pharynx resulted in fatality [50].

Presented clinical signs of infected birds depend on the species and age of the host, virus strain and also the pathophysiological changes in the respiratory, digestive, urinary, nervous and reproductive systems [44, 51, 52]. Hence, avian influenza virus pathobiology varies among strains and the host species. Therefore, pathobiology characters of new avian influenza virus are important to control the outbreaks and understand the epidemiology of this disease [53].

Clinical signs and pathological features of H5N1 in layer chickens from East Java, Central Java, West Java and Yogyakarta during 2003–2005 outbreaks have demonstrated depression, loss of appetite, neurologic disorder, respiratory disorder, egg production drop and diarrhoea [54]. These clinical signs were similar to previously described infections naturally or experimentally with highly pathogenic avian influenza virus in domestic poultry [44, 45, 51, 55, 56].

On post-mortem examination of infected chicken showed severe subcutaneous haemorrhages, oedema in the wattles, head, neck, and the leg shanks appeared haemorrhages [55]. However, Mutinelli et al. [45] and Elbers et al. [57] also described peritonitis; haemorrhage, enlarged and hardened of pancreas; enlarged

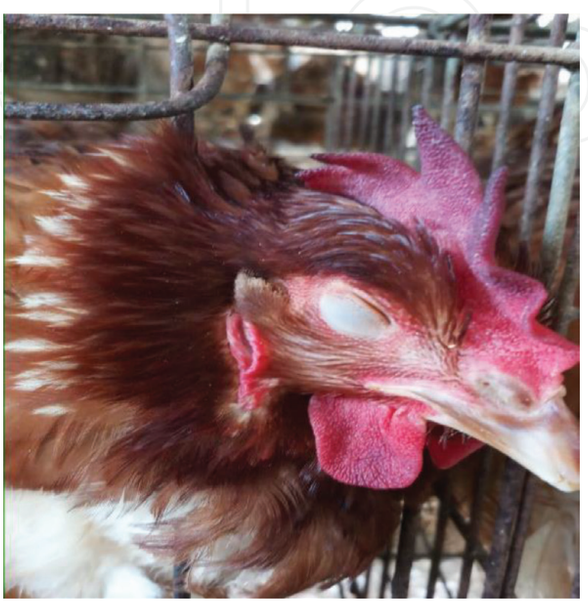

Figure 4.
Latest cases of Avian Influenza: Cyanotic wattles (Courtesy: Dr. Sitarina Widyarini).

Figure 5.
Latest cases of Avian Influenza: Brain congestion (Courtesy: Dr. Sitarina Widyarini).

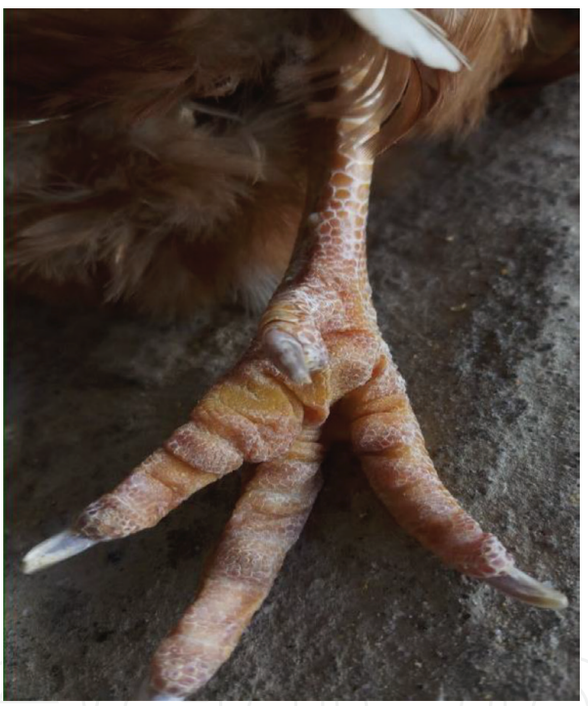

Figure 6.
Latest case of Avian Influenza: Haemorrhages feet (Courtesy: Dr. Sitarina Widyarini).

with whitish and dark brown haemorrhage of liver areas. In a few cases, proventriculus and ventriculus showed petechial haemorrhages [45, 55, 57], haemorrhages of comb and wattles, ecchymose haemorrhages in the skin of the breast and abdomen [47]. Similar lesions such as cyanotic wattles, swollen head and comb, haemorrhages in the skeletal muscles, abdominal fat, proventriculus and feet were also observed in chicken during 2003–2005 Avian Influenza outbreaks in Indonesia [54]. Furthermore, in layer chickens, haemorrhagic ovary and atrophy oviduct were also found [54, 56, 58]. Similar findings in mute and whooper swans infected by HPAI, was showing coalescent haemorrhages with necrosis in the pancreas [59, 60], kidney enlargement yet elastic without deposits of uric acid [45].

Recent case in layer chicken of 40 weeks from East Java (August, 2018) with cyanotic wattles (**Figure 4**), brain congestion (**Figure 5**), haemorrhages in the feet (**Figure 6**) and proventriculus (**Figure 7**), haemorrhages and adhesion

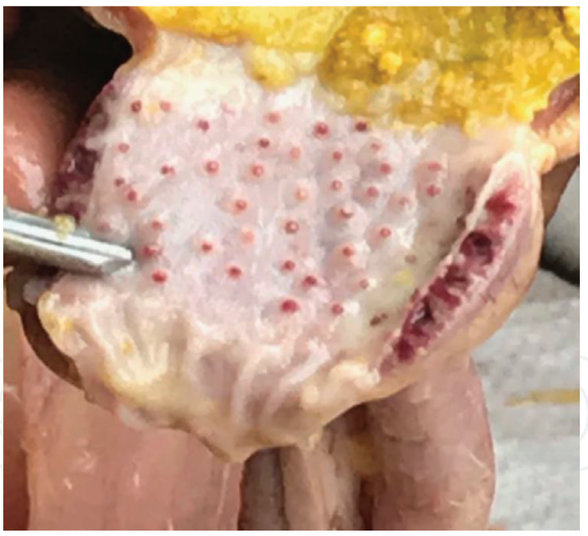

Figure 7.
Latest case of Avian Influenza: Proventriculus Haemorrhages (Courtesy: Dr. Sitarina Widyarini).

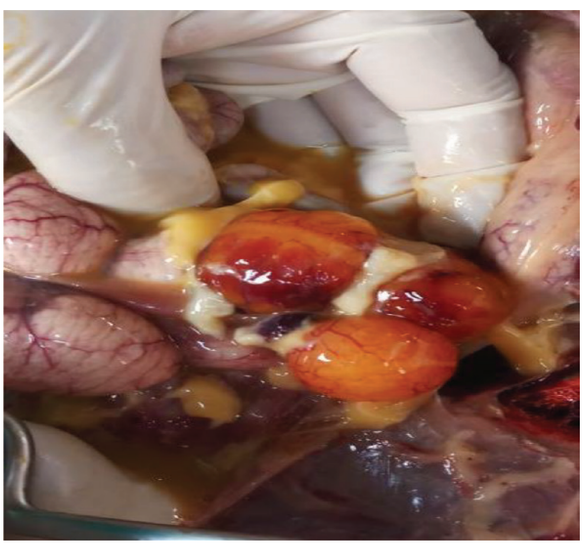

Figure 8.
Latest case of Avian Influenza: Ovarial Haemorrhages (Courtesy: Dr. Sitarina Widyarini).

between ovarian follicles (**Figure 8**), haemorrhage of abdominal fat (**Figure 9**), haemorrhage of pectoral muscles (**Figure 10**), swollen and oedematous kidney (**Figure 11**). The farm experienced 20% mortality rates within 3 weeks and the egg production dropped by 18% suddenly in 5 days. Vaccination for avian influenza H5 was done at 14 weeks of chick age. Molecular identification was confirming H5 subtype. In a few cases, virus can be isolated from properly vaccinated flock [61].

Histopathological findings of HPAIV-infected chicken and turkey were dominated by acute haemorrhages (skin, under serous membrane, mucosae and pectoral muscles), oedema (skin of head, neck, legs and lungs) and necrosis (skin, pancreas, spleen and heart) [42, 46, 49, 55, 62–66]. The comb and wattles showed markedly severe cellulitis associated with congestion, oedema and mild heterophilic infiltration in the dermis and subcutis [55]. Lymphocytic meningo-encephalitis and meningo-encephalomyelitis with multifocal gliosis, degeneration of neuron, necrosis and neuronophagia, as well as mild-to-moderate perivascular cuffs, with predominance of macrophages and lymphocytes in both grey and white matter in the majority of brain region [60, 67, 68]. Necrosis with focal lymphohistiocytic

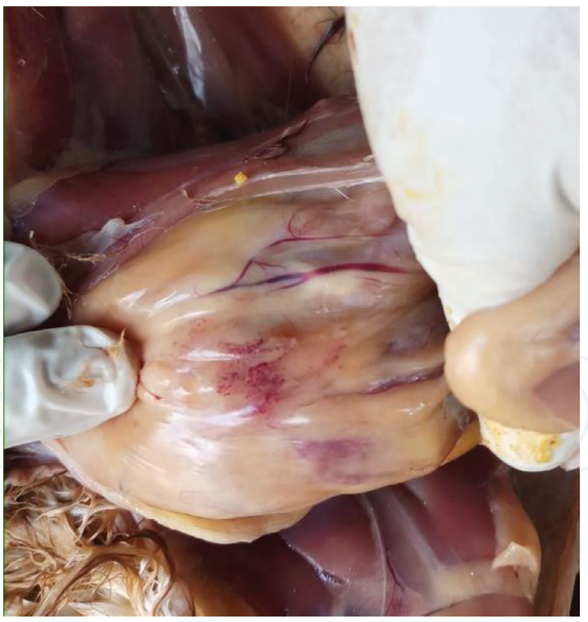

Figure 9.
Latest case of Avian Influenza: Abdominal fat haemorrhages (Courtesy: Dr. Sitarina Widyarini).

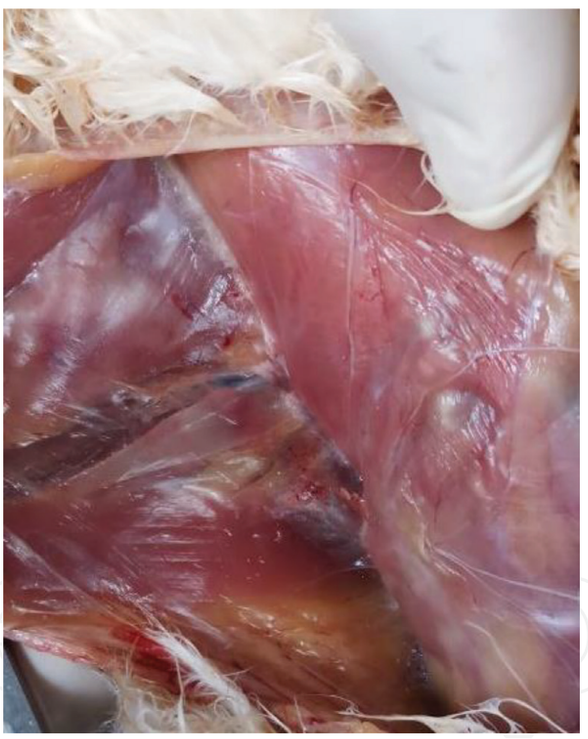

Figure 10.
Latest case of Avian Influenza: Pectoral muscles haemorrhages (Courtesy: Dr. Sitarina Widyarini).

infiltration in the myocardium, focal necrosis in the pancreas and other organs (e.g. lungs, lymphatic organs and skeletal muscles) are defined as important histopathological lesions [58, 60, 68].

Histological lesions associated with the presence of viral antigen were observed in the tissue of infected chickens. Several studies have observed intranuclear and intracytoplasmic viral antigens distribution at surrounding tissues of parenchymal myofibres and capillary endothelium of the heart, hepatocytes and sinusoidal endothelium of the liver, pulmonary endothelium, pancreas, kidney, central nervous system, leukocytes of the Peyer's patches, bursa, epithelium of the adrenal glands, renal tubules and pancreatic acini [44, 55, 69, 70].

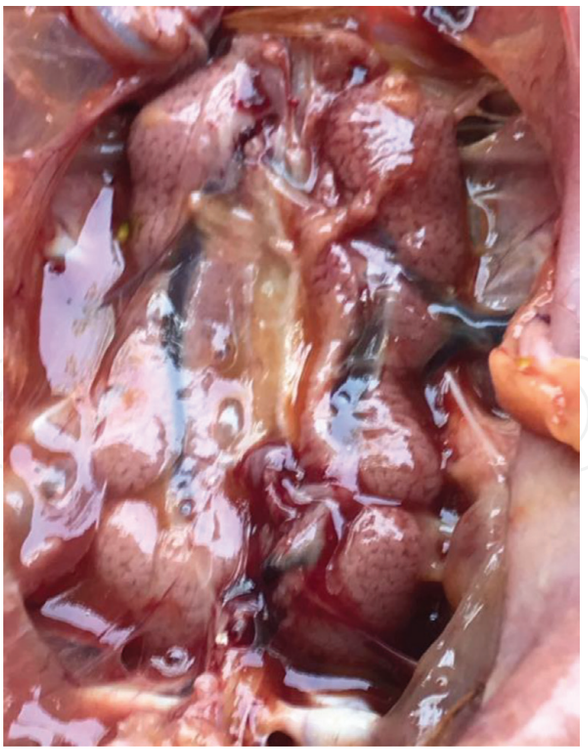

Figure 11.
Latest case of Avian Influenza: Swollen and oedematous kidney (Courtesy: Dr. Sitarina Widyarini).

5. Conclusion

Vaccine application and stringent biosecurity practices helped to suppress the viral load in the flock. As consequences, the morbidity and mortality rate is suppressed, the presentation of clinical signs is milder although the gross pathology features remained consistent. The introduction of H9N2 has initiated the new threat. Egg production drop is today mainly observed as an indication of infection regardless of the virus subtype, although as the latest published active surveillance data (2012–2013) continued blaming H5N1. The masking effect of partial low-level herd immunity may be responsible for the phenomenon.

Virus isolated from chicken with both specific and non-specific lesion between 2003 and 2006 showed high pathogenic avian influenza virus based on molecular marker analysis. Although vaccination has been applied, full viral characterisation continues, evaluation of antibody protective response after vaccination and differentiation between vaccinated and infected birds is needed. Cartography surveillance of avian virus is importantly required to understand cross-immunity of latest strains to use as vaccine seeds. Antigen panel is a must in order to predict future outbreak. Enforcement on regulation for live birds market (LBM) is a must, considering massive human death in China of novel reassortant virus. In addition, wild bird migration from Asia to high densities poultry farms population in Java could increase reassortment rate of circulating virus. Furthermore, the finding of A(H3N8) may trigger novel reassortant virus strain with zoonotic potential. Although, human cluster, Tangerang and Karo, is required for further research since the cases occurred only between people with genetic relation.

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Conflict of interest

The authors Khrisdiana Putri, Sitarina Widyarini, Sugiyono and Widya Asmara have no conflict of interest to declare.

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