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Research Article

Pulmonary Pathognomonic Lesions of Anatomical Pathology and Histopathology for Avian Influenza Virus in Normal Looking-Avian Influenza Virus Infected Layer Chickens with Decreased Eggs Production) Based on the Immunohistopathological Approach

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Abstract: The outbreak of avian influenza virus (AIV) has spread in almost all provinces in Indonesia. Recently, it is reported, that layer chickens look healthy have decreased egg production. At necropsy, anatomical pathological lesions, such as pulmonary petechial and/or linear hemorrhages were observed. In chickens, AIV is endotheliotrophic, therefore a research needs to be done to evaluate whether the typical pulmonary hemorrhagic lesions is an AIV pathognomonic pathological lesions that can be used as a confirmative diagnosis for rapid and accurate AIV diagnosis. Twenty layer chickens looked healthy were used. All were necropsied and observed anatomical pathological lesions in the lungs. The lungs were then made histopathologically with routine staining of hematoxylin and eosin. The pulmonary anatomical pathological and histopathological lesions due to AIV infection were confirmed by applying immunohistopathological streptavidin biotin (IHC SB) with polyclonal antibody anti AIV nucleotrotein. The paraffin blocks of the lungs that are AIV immuniopathological positive were recut and stained with a routine hematoxylin and eosin stain. Histopathological lesion(s) of the hemorrhagic lungs were examined under the microscope, and were analyzed descriptively**.** In the present study, all layer chickens which were necropsied, had anatomical pathological lesions of petechial and / or linear hemorrhages and histopathological lesions of congestion and severe, diffuse hemorrhages in the lungs. It was concluded that pathological lesions in the lungs are pathognomonic in chickens infected with AIV.

Keywords: AIV, pulmonary, pathognomonic, layer chickens, healthy

INTRODUCTION

In Indonesia, the poultry industry sector, especially commercial layer and the breeding layer farms show a significant increase lately, and there is still potential for further development. Infectious disease in poultry is the biggest threat in those poultry industries and will be able to impact on the hampered growth rate of them.

Avian influenza virus (AIV) has become epidemic in poultry in the world and has repeatedly destroyed the poultry industry that existed throughout the world in the past (Capua and Alexander, 2004). In the case of outbreaks of viral diseases caused by infectious and infective AIV in chickens, AIV can be grouped into 144 subtypes based on glycoprotein surface antigens, namely hemagglutinin and neuraminidase (Fouchier *et al*., 2005). All isolates (AIVs) belonging to the same AIV subtype have different pathogenic abilities. Some AIVs have low or low pathogenic properties known as low pathogenic AIV (LPAI), and some have high or high pathogenic properties, known as highly pathogenic AIV (HPAI) (Capua and Alexander, 2004). AIV-infected chickens exhibit very variable clinical symptoms, ranging from the absence of clinical symptoms, chickens appear normal, healthy or subclinical to a decrease in egg production, even deadly (Easterday *et al*., 1977; Wasito *et al*., 2014; Ekaningtias *et al*., 2017). In LPAI-infected chickens, clinical symptoms that arise depend on the species and age of poultry, AIV strains and subtypes, and the presence / absence of secondary bacterial infections, especially *Escherichia coli* (Webster and Kawaoka, 1988; Easterday *et al*., 1997). Sometimes, chickens will die without showing any clinical symptoms. In poultry, the HPAI subtype is highly infective causing severe hemorrhages in various organs, including: lungs, trachea, brain, heart, flesh muscle, egg

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follicles and kidneys (Yunita *et al*., 2017; Lu *et al*., 2019). Meanwhile, LPAI infections in poultry, in general, will not cause clinical symptoms (subclinical). This means that chickens infected with LPAI appear normal (healthy) and anatomic pathological lesions only occur, especially in the respiratory tract and / or digestive tract in the form of spotted and / or linear hemorrhages. AIV strains in poultry that circulate in the field and in the poultry industry are LPAI. These LPAI strains are capable of acting as a source of AIV transmission in other sensitive birds continuously and capable of mutation into HPAI so they can be fatal in infected chickens (van Dijk *et al*., 2018). Pathological and immunopathological tests are expected to be developed and applied in the determination of early diagnosis of LPAI quickly in healthy-looking poultry. Thus, the government's efforts in overcoming, including control, prevention and eradication of AIV in the field can be implemented optimally.

Avian influenza infections caused by avian influenza virus (AIV) in poultry are acute with very significant morbidity and mortality rates. According to OIE (2008), AIV is included in List A with a high mutation rate because it has antigenic drift and genetic shift (genetic reassortment) properties (CDC, 2017). The significance of the antigenic drift phenomenon in AIV outbreaks is that LPAI strains can turn into highly virulent HPAI strains due to mutations in only one amino acid in the glycoprotein HA or H (hemagglutinin) sections. This can occur because AIV does not have the ability of a genetic mechanism to recognize and correct the RNA genome if there is a misprint of RNA during AIV replication. RNA genetic errors during irreversible AIV replication will result in genetic makeup and the formed AIV strains are replaced by new antigenic AIV variants that have severe or high pathogenicity, known as highly pathogenic AIV (HPAI). Meanwhile, genetic reassortment is an AIV genetic exchange phenomenon that can occur in certain hosts, especially pigs. Pigs are a host that is sensitive to the possibility of a mixed infection between AIV and the mammalian flu virus so that it can produce a new form of flu virus subtype that can be fatal to humans if infected with the new form of the virus (WHO, 2017). Besides pigs, humans can also act as a genetic reassortment phenomenon. Avian influenza virus H5N1 subtype in poultry is the most important to note because the AIV H5N1 is easily and quickly mutated from LPAI to HPAI. In addition, AIV H5N1 has been known to have the ability to receive flu virus genes from other animal species and has the ability to infect and cause severe flu in humans (Webster *et al*., 1992; Wood *et al*., 1993). The phenomenon of genetic exchange occurs because of the fact that AIV has segmented genomes (Car and Toner, 1982).

MATERIAL AND METHODS

Organs Samples

In the present study, 20 samples were used in the form of lung tissue which were AIV target organs (Wasito *et al*., 2018; Susiani *et al*., 2019) derived from layer chickens that looked normal (healthy) or showed no clinical symptoms, except experienced decreased egg production by ± 10-25%. At necropsy, the lungs are observed for presence or absence of anatomic pathological lesions, in the form of spotted and/or linear hemorrhages. The lungs (20) based on positive immunopathological test results, subsequently, the paraffin block of the lungs are re-cut, and processed for histopathological preparations with routine staining of hematoxylin-eosin.

A Routine Haematoxylin-Eosin Staining

For routine histopathological staining with hematoxylin-eosin stain, lung paraffin blocks originating from layer chickens (20) which are immunopathologically AIV positive, were collected and subsequently re-cut with microtome thickness of 3-5 µm. For routine staining of hematoxylin-eosin, 3-5 µm tissue (lung) preparations were deparafinized with xylene 3 times, 5 minutes each, ethanol concentration decreases (absolute and 95% 5 minutes respectively), washed with distilled water and PBS each 1 x 2 minutes, dipped in Harris-hematoxylin 20 minutes, washed distilled water, acid dipped 2-3 alcohol dip, washed distilled water 1 minute and 15 minutes, dipped in eosin solution 2 minutes, put in ethanol 96% 2x each 3 minutes, absolute ethanol 2x each 3 minutes, washed xylene 2x, 5 minutes each. Next, the histopathological preparations of the lungs were given a glycerol adhesive medium and covered with a glass cover to be observed under a microscope. The anatomic and histopathological pathological lesions of the lungs were analyzed descriptively.

RESULTS AND DISCUSSION

In the year 208-2019, even until 2020 in several regions in Indonesia, especially in the Provinces of East Java, Central Java and West Java, many layer chicken farmers whose chickens decreased egg production ± 10-25%, and the cause not yet known. Here, it is reported, that based on the results of the present study, with a streptavidin biotin immunohistopathological (IHC SB) with antibody anti nucleoprotein avian influenza virus (AIV) test, the chickens were proven to be infected with a low pathogenic AIV (Figure 1). Even so, the prevalence of AIV in poultry, including ornamental birds (Zulfikhar *et al*., 2019) and allowing repeated outbreaks of AIV (Wasito *et al*., 2018) in poultry, resulted in AIV needing to get serious attention. In addition, given that infection caused by AIV in poultry can be acute with a very significant morbidity and mortality rate in poultry due to the Antigenic drift properties of AIV (Kang *et al*., 2017; Li *et al*., 2018), and the possibility of causing serious public health disorders due to the nature of genetic shift (genetic reassortment) AIV. Live poultry play an important role as a cause of AIV outbreaks continuously because poultry as the main source that plays a role in AIV transmission so that outbreaks occur (Offeddu *et al*., 2016). Thus, a test is needed that is able to confirm the potential presence and possibility of AIV spread in poultry.

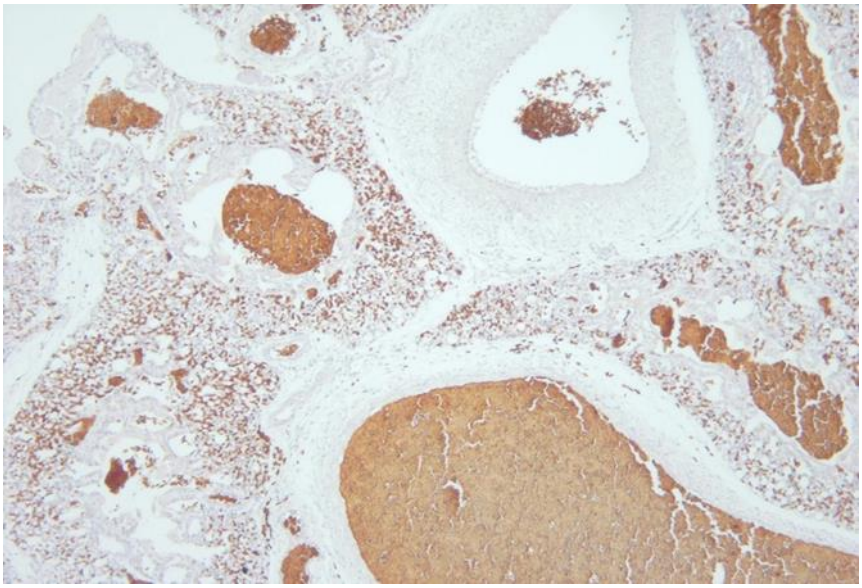

Figure 1. Immunohistopathological features of streptavidin biotin antibody anti-nucleoprotein avian influenza virus (AIV) in the lungs of layer chickens that appear normal (healthy) or show no clinical signs, except have decreased egg production ± 10-25%. AIV-infected lungs show a brownish color in the parabronchi, blood vessels and lung parenchyma (Streptavidin and biotin, 250x.).

In the present study, lung tissue is used as AIV target organ (Wasito *et al*., 2018; Susiani *et al*., 2019) derived from layer chickens that appear normal (healthy) or show no clinical symptoms, except for decreased production eggs ± 10-25%. At necropsy, the lungs show anatomical pathological lesions in the form of egg follicle atrophy (Figure 2) and spotted and / or linear hemorrhages in the lungs (Figure 3). On histopathological examination with routine staining of hematoxylin and eosin, the lungs look congested and are heavily linear and hemorrhagic (Fig. 4). AIV is a global threat and outbreaks occur not only in poultry, but can also in humans. However, global efforts to monitor AIV genetic diversity that circulate in nature have not been completely carried out (Latorre-Margalef *et al*., 2014).

The results of the present study indicate that AIV was found to have a low level of virulence or low pathogenic avian influenza (LPAI) due to anatomical pathological lesions seen only in the respiratory tract (lungs) and egg follicles. In general, AIV has a low virulence rate (LPAI). However, LPAI can mutate (antigenic drift) to become virulent AIV or highly pathogenic AIV (HPAI) which is fatal in poultry. In addition, two AIV subtypes, namely H5N1 (Yang *et al*., 2016) and H7N9 (Lai *et al*., 2013), at present, pose a threat to AIV pandemics in humans due to their ability to do host reaction (genetic shift or genetic reassortment)so that it can infect humans (Poovorawan *et al*., 2013).

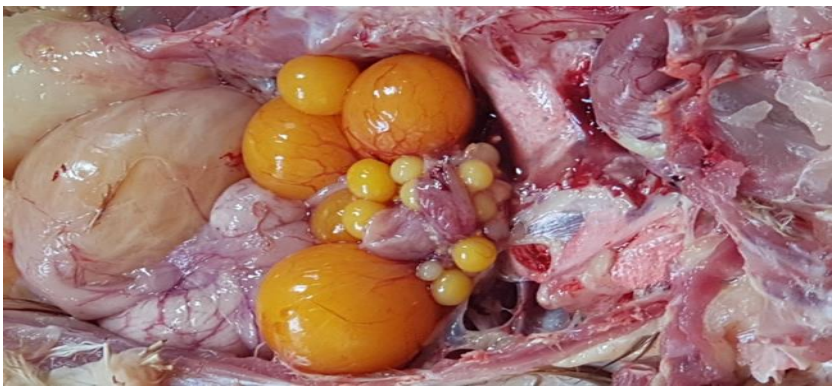

Figure 2. An overview of anatomic pathological lesions of egg follicles of layer chickens that appear normal (healthy) or show no clinical symptoms, except have decreased egg production ± 10-25%. which with the immunohistopathological test of streptavidin biotin antibody anti-nucleoprotein avian influenza virus (AIV) in AIV infected lungs. Egg follicles atrophy.

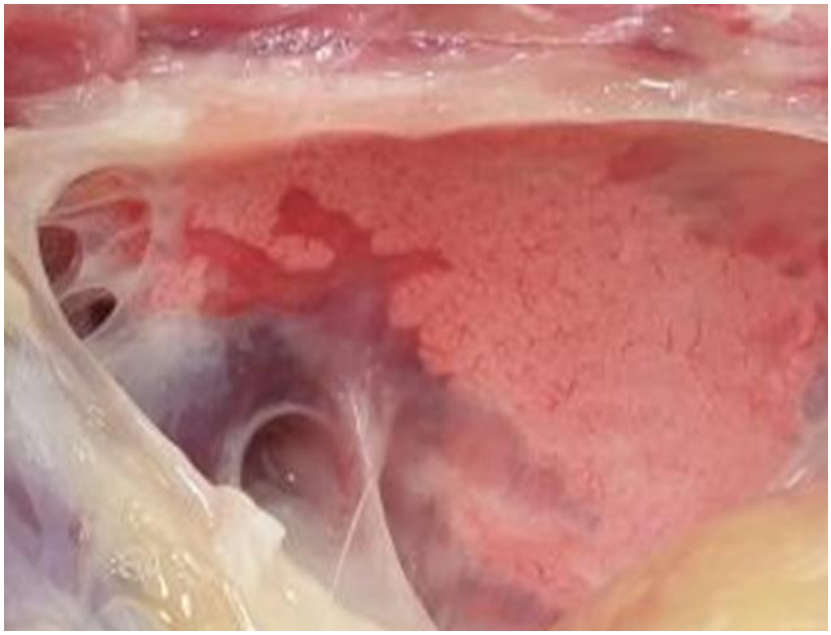

Figure 3. Picture of anatomic pathological lesions of the lungs of layer chickens that appear normal (healthy) or do not show clinical symptoms, except have decreased egg production ± 10-25% which tested positive for AIV immunohistopathology. The lungs are swollen, blurred and there is congestion and severe linear and spotted hemorrhages.

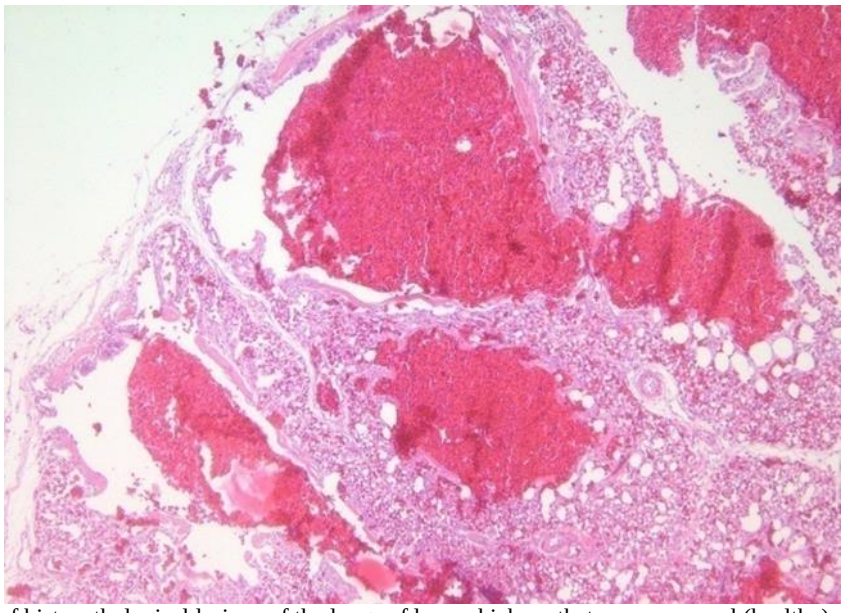

Figure 4. Picture of histopathological lesions of the lungs of layer chickens that appear normal (healthy) or show no clinical symptoms, unless they have decreased egg production ± 10-25% which with the immunohistopathological test of streptavidin biotin antibody anti-nucleoprotein avian influenza virus (AIV) in the lungs are positively infected with AIV. There is congestion and severe diffuse hemorrhages in the pulmonary vessels and parencghyma (Hematoxylin and eosin, 500x.).

Histopathological lesions in poultry caused by AIV H5 subtype of pathogenic malignancy or highly pathogenic avian influenza (H5 HPAI) have been reported by Pantin-Jackwood *et al*. (2017), which indicates that HPAI is capable of multiplication in various organs and cell types so resulting in necrosis of cells, tissues or organs. Nevertheless, the inflammatory reaction due to infection with various AIV HPAI subtypes in poultry is very different from one AIV subtype with other AIV subtypes. Thus, confirmation of the presence of pathognomonic histopathological lesions in each AIV HPAI subtype is indispensable for the confirmation of the diagnosis.

CONCLUSION

Based on the results of the present study, it is evident that the pathognomonic pathological lesions are petechial and / or linear hemorrhagic lung in layer chickens infected with avian influenza virus that appear normal (healthy) or do not show clinical symptoms, except have decreased egg production ± 10-25%

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