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Research Article

Pathognomonic Pathological Lesion of Digestive Tracts in Chickens That Are Immunopathologically Positive of Avian Influenza and Newcastle Disease Viruses

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Abstract: Bird flu or avian influenza (AI) and *tetelo* disease or Newcastle disease (ND), each of which is caused by AI virus (AIV) and ND virus (NDV) are the most important viral disease in poultry that has been widely reported in the world. AIV and NDV have long been known to circulate in poultry in Indonesia. Nonetheless, detailed information on pathognomonic pathological lesions of the two viruses in the digestive tract has never been studied and reported. AIV and NDV viruses in birds have similar clinical signs, namely torticollis and curled toe paralis, accompanied by hemorrhagic pathological lesions in digestive tract. In the present study, 20 layer chickens previously showed clinical neurological symptoms, namely torticollis and curled toe paralysis, and focal necrotic hemorrhagic lesions of the gastrointestinal tract were used. They are originating from field cases in the poultry farming industry based on the positive results of immunopathological immunohistochemical examination of streptavidin biotin, anatomical pathological lesions and histopathological features were observed and examined. Based on the results of this study, pathognomonic anatomical pathological and histopathological lesions of AIV and/or NDV cannot be determined based solely on the results of poultry necropsy in the field. In the case of AI and ND diseases, confirmation of laboratory diagnosis is needed, especially immunopathological immunohistochemistry as an indicator whether chicken is infected with AIV and / or NDV in order to properly, accurately and optimally overcome it.

Keywords: digestive tract, AIV and / or NDV, anatomical pathological lesions, histopathologic lesion, necropsy.

NOVELTY

Based on the results of the present study, confirmation of the diagnosis of AI and / or ND in poultry can be done quickly, precisely and accurately, directly as well in the field at the time of necropsy.

INTRODUCTION

Avian influenza virus (AIV) and Newcastle disease virus (NDV) are capable of causing diseases outbreaks in various species of birds, especially layers, broiler and villages chickens. Avian influenza virus is included in the Orthomyxoviridae family, while NDV is one of the avian paramyxovirus serotype-1 (APMV-1) from the family Paramyxoviridae (Swayne DE and King DJ, 2003). The severity of clinical signs and pathological lesions in birds infected with AIV or NDV varies, from death to persistent infection (birds do not show any clinical signs, normal or healthy) (Alexander DJ and Senne DA, 2008; Lamb RA and Griffit PD, 2013).

And, both AI and ND viruses can be transmitted to humans or are zoonotic. In mammals and humans, NDV can cause mild inflammation in the eyes (uveitis) (Obaldia III N and Hanson RP, 1989), and AIV can cause respiratory problems (Anonymous, 2010). Significant mortality in chickens can be caused by highly pathogenic avian influenza virus (HPAI) and velogenic NDV (vNDV). Thus, the presence of vNDV and AIVsubtipe HPAI, especially H5 and H7 viruses are a major concern of OIE (Office of International des Epizooticae) (Swayne DE and King DJ, 2003; Lee CW *et al.*, 2005). NDV genotypes are serologically the same, so low pathogenicity NDV genotypes are used as NDV seeds in the manufacture of ND vaccines to neutralize natural infections of pathogenic NDV in

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poultry. In contrast, the AIV genotype is very different between one AIV subtype and the other AIV subtype in terms of immunogenicity, so that there is no cross protection between AIV viruses that differ in terms of surface glycoproteins, ie hemagglutinin (HA) (Anonymous, 2010).

Wild birds that migrate and the traffic of birds (chickens) trade live are thought to be the main source of transmission of AIV and / or NDV so that the two viruses are endemic to almost the entire world. However, especially for AIV, ducks and geese are considered a source of important AIV transmission in poultry in the AIV endemic areas (Swayne DE and King DJ, 2003). Commercial chicken is a poultry production sector that is often infected with AIV and / or NDV in developing countries, including Indonesia (Alexander DJ, 2001).

Viral Newcastle disease has circulated in Indonesia (Darminto and Ronohardjo P, 1996; Wasito R *et al.*, 2016a, b; Wasito R *et al.*, 2018a, b), and low pathogenic avian influenza virus (LPAI) and highly pathogenic avian influenza virus (HPAI) has been prevalent in Indonesia (Sedyaningsih ER *et al.*, 2006; Wuryastuti H and Wasito R, 2013; Wasito R *et al.*, 2014a, b, c; Wasito R *et al.*, 2015; Wasito R *et al.*, 2016a, b; Tyasasmaya T *et al.*, 2016). In general, AIV and / or NDV in Indonesia are endemic to commercial poultry (Wasito R *et al.*, 2016a, b; Wasito R *et al.*, 2018a, b).

Nevertheless, in cases of NDV and / or AIV outbreaks in the field, clinical signs and anatomical pathological lesions between AIV and NDV are difficult to distinguish. Clinical signs and pathological lesions of the digestive tract between the two viruses are similar, including: torticollis and curled toe paralysis, and pathological lesions in the form of hemorrhagic gastrointestinal tract (Wuryastuti H *et al.*, 2005a, b; Wasito R *et al.*, 2016a, b; Wasito *et al.*, 2018a, b). Thus, based on the results of the present study, it is expected to be known and determined pathognomonic pathological lesions in the gastrointestinal tract that can distinguish between the two viral diseases so that it can be applied to confirm both viral diseases when necropsy is carried out in poultry in the field.

MATERIAL AND METHODS

Organ Samples

In the present study, 20 organ samples (the gastrointestinal tracts) with focal necrotic hemorrhagic lesions originating from layer chickens were used. All the chickens were shown to be positively infected with AIV and NDV by using immunopathologic immunohistochemical streptavidin biotin (IHC SB) test. The digestive tracts of paraffin blocks originating from the positive IHC SB preparation were collected, then they were processed for histopathological preparations with the routine staining of hematoxylin-eosin.

Pewarnaan Histopatologis Rutin Hematoksilin-Eosin

Untuk pewarnaan rutin histopatologis dengan pewarnaan hematoksilin-eosin, blok parafin saluran pencernaan yang berasal dari sediaan imunopatologis IHK SB AIV dan NDV positif akan diambil dan selanjutnya akan dipotong ulang dengan mikrotom ketebalan 3-5 µm. Untuk pewarnaan rutin hematoksilin-eosin, sediaan 3-5 µm jaringan (saluran pencernaan) di-deparafinisasi dengan xilen 3x, masing-masing 5 menit, etanol konsentrasi menurun (absolut dan 95% masing-masing 5 menit), dicuci aquades dan PBS masingx2 1 menit, dicelupkan ke dalam Harris-hematoksilin 20 menit, dicuci aquades, dicelup *acid alcohol* 2-3 celupan, dicuci aquades 1 menit dan 15 menit, dicelupkan ke dalam larutan eosin 2 menit, dimasukkan ke dalam etanol 96% 2x masing-masing 3 menit, etanol absolut 2x masing-masing 3 menit, dicuci xilen 2x, masing-masing 5 menit. Selanjutnya, sediaan histopatologis saluran pencernaan tersebut akan diberi medium perekat gliserol dan ditutup dengan gelas penutup untuk diamati di bawah mikroskop.

Routine Histopathological Staining of Hematoksilin-Eosin

For routine histopathological staining with hematoxylin-eosin, block digestive tract paraffins derived from immunopathological immunohistochemical streptavidin biotin preparations for positive AIV and ND were collected and were then cut back with a 3-5 µm microtome thickness. For routine staining of hematoxylin-eosin, preparations of 3-5 µm tissues (digestive tracts) were deparafinized with xylene 3x, 5 minutes each, rehidrated with ethanol concentration decreases (absolute and 95% each 5 minutes), washed with distilled water and PBS each x2 1 minute, dipped in Harris-hematoxylin 20 minutes, washed distilled water, dyed acid alcohol 2-3 dyes, washed distilled water 1 minute and 15 minutes, dipped in 2 minutes eosin solution, put in ethanol 96% 2x each 3 minutes, 2x absolute ethanol 3 minutes each, washed 2x xylene, 5 minutes each. Furthermore, the histopathological sections of the digestive tracts were given an adhesive medium of glycerol and covered with a lid to be observed under a microscope.

Statistical Analysis

Anatomical pathological lesions and histopathology of the gastrointestinal cell mucosa derived from the results of immunopathological immunohistochemistry streptavidin biotin AIV and NDV positive were analyzed descriptively.

RESULTS

In the present study, 20 chickens (layer, broiler and non-race) were used, and it was proven, that chickens showing clinical signs of head spinning

irregularly up, down, left and right (torticollis), and swollen and convulsions of the legs (curled toe paralysis) and pathologic focal necrotic hemorrhagic lesions of the gastrointestinal tract (Figure 1). Furthermore, in the present study, it was determined whether anatomical pathological lesions and hemorrhagic histopathology in the digestive tract of chickens are pathognomonic due to avian influenza virus (AIV) and / or Newcastle disease virus (NDV) infection by applying immunopathological immunohistochemical streptavidin biotin (IHC SB).

In this study, at the time of necropsy, it was seen pathological lesions in the proventriculus in the form of petechial hemorrhages (spotted hemorrhage) which differed in severity in all chickens that were necropsied (Fig. 2-3). Whereas most ileum had diffuse multifocal hemorrhages pathologically (Fig. 4-5). Only one chicken showed pathological lesions of focal necrotic hemorrhages in the upper gastrointestinal tract, especially the ileum. In the lower part of the digestive tract (cecum) there were no visible macroscopic lesions of hemorrhages.

In the previous study, the digestive tract tissue (proventriculs and ileum) which tested single IHC SB is positively infected with AIV (polyclonal antibody anti-nucleoprotein AIV) and NDV (polyclonal antibody anti hemagglutinin-neuraminidase / HN NDV). AIV and NDV antigens are located mainly in the nucleus and cytoplasm of proventricular and ileal epithelial cells that had hemorrhagic necrosis (Fig. 6-7) (Wasito et al., 2016; Wasito et al., 2018).

DISCUSSION

Diagnosis of AIV and NDV in poultry, in general, is based on virus isolation and identification. However, confirmation of the two diagnostic approach methods takes a relatively long time, and at least, a definitive diagnosis can be delayed more than 2 weeks (Swayne DE and King DJ, 2003). Reverse transcriptase polymerase chain reaction (RT-PCR) (Tyasasmaya T *et al.*, 2016), multiplex RT-PCR (Wasito R *et al.*, 2014a, b, c; Wasito et al., 2015) and real-time PCR have also been developed and applied to confirm the diagnosis of AIV and / or NDV in poultry. Molecular testing of RT-PCR, mRT-PCR and rt-PCR require strict laboratory infrastructure facilities, namely biosafety level II or III and relatively expensive fine chemistry.

Figure 1. Two layer chickens infected naturally with avian influenza virus and Newcastle disease virus. Torticollis and curled toe paralysis are seen.

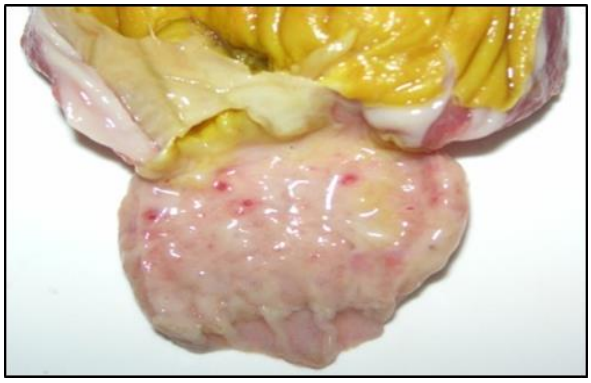

Fig. 2. Anatomical patological lesion in proventriculus of a layer chicken infected naturally with avian influenza and Newcastle disease viruses. Notice anatomical pathological lesions of petechial hemorrhages with a different degree of severity.in proventriculus.

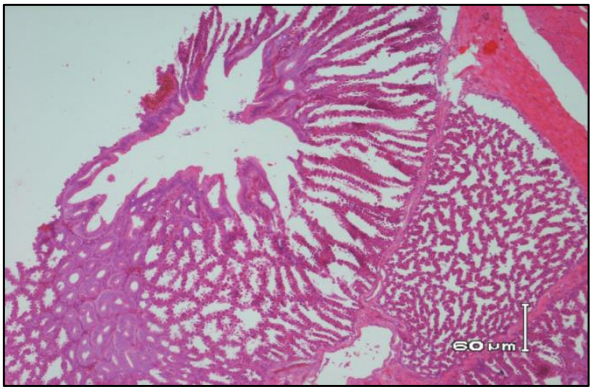

Fig. 3. Histopathological lesion in proventriculus of a layer chicken infected naturally with avian influenza and Newcastle disease viruses. Notice histopathological lesions of petechial hemorrhages in epithelial cells (Hematoksilin and eosin, 250x.).

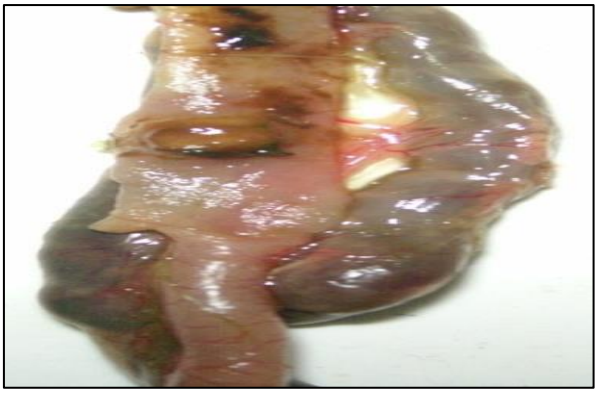

Fig. 4. Anatomical patological lesion in ileum of a layer chicken infected naturally with avian influenza and Newcastle disease viruses. Notice a mkroscopic lesion of focal necrotic hemorrhages.

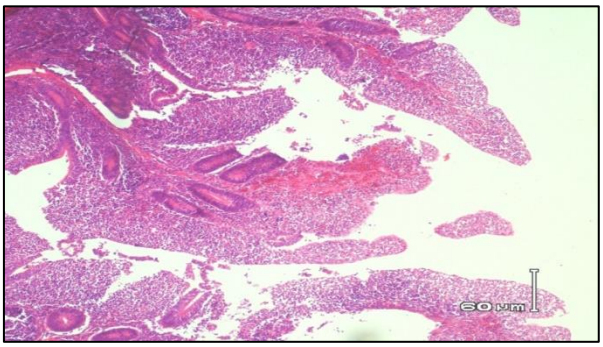

Fig. 5. Histopathological lesion in ileum of a layer chicken infected naturally with avian influenza and Newcastle disease virus. Notice multifocal and diffuse hemorrhages in epithelial cells (Hematoksilin and eosin, 250x.).

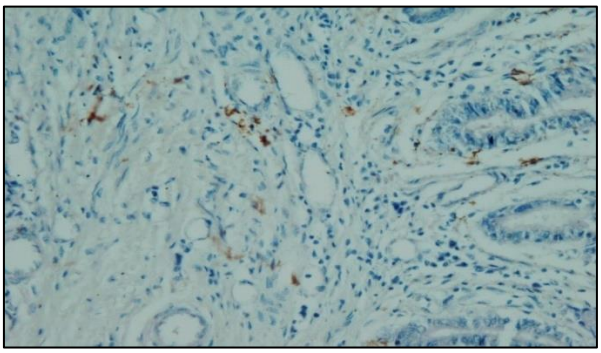

Fig. 6. Ileum tissue of a layer chicken naturally infected with avian influenza virus (AIV) with immunopathological immunohistochemical streptavidin biotin using polyclonal antibody anti AIV nucleoprotain. (NP). Notice NP AIV looks brownish coloration (Streptavidin biotin, 500x.).

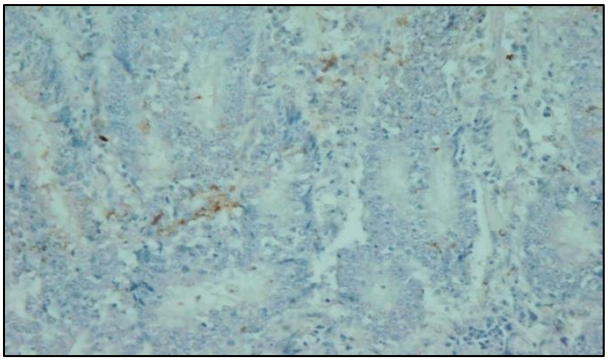

Fig. 7. The ileum tissue of a layer chicken originating from the same duodenal tissue as in Figure 1, immunopathological immunohistochemical streptavidin biotin using polyclonal antibody anti NDV hemagglutinin-neuraminidase / HN NDV. Duodenum is also infected with Newcastle disease virus (NDV). Antigen HN NDV looks brownish coloration (Streptavidin biotin, 500x.).

Viral isolation and RT-PCR tests can result in laboratory and environmental contamination allowing the spread of AIV and/or NDV infections in poultry and humans (Wasito *R et al.*, 2014a, b, c). Immunopathological immunohistochemistry (IHC) has been applied extensively in confirmation of diagnosis and studies related to tropism and viral antigen distribution in virus-infected tissues (Wasito R and Wuryastuti H, 2016; Wasito *et al.*, 2016a, b; Wasito *et al.*, 2018a, b). IHC method is environmentally friendly, fast and accurate early diagnosis techniques, and relatively inexpensive costs (Offedu V *et al.*, 2016; Tyasasmaya T *et al.*, 2016) so as to minimize the incidence of AIV and/or NDV outbreaks needs to be applied optimally in Indonesia.

As is known, that AIV and NDV are viral diseases in poultry that can result in relatively high mortality and morbidity (Belak S *et al.*, 2009; OIE, 2012). Outbreaks of ND and AI in poultry in poultry breeding farms show that in poultry infected with AIV and / or NDV have similar clinical signs and pathological anatomical lesions. These clinical signs include torticollis and curled toe paralysis. At the time of necropsy, the digestive tract, especially the proventriculus, duodenum, jejunum ileum had the same lesions, namely hemorrhages in the form of petechial hemorrhages and focal necrotic hemorrhages (Wuryastuti H *et al.*, 2005a, b; Wasito R *et al.*, 2016a, b; Wasito R *et al.*, 2018a, b). Anatomical pathological lesions seen in the digestive tract of birds infected with AIV and / or NDV are highly dependent on viral strains, it is even possible, and that in birds infected with AIV and / or NDV does not show any anatomical pathological lesions of hemorrhages in the digestive tract (Hongxin L *et al.*, 2018).

Pathognomonic anatomical pathological and and histopathological lesions of AIV and/or NDV cannot be determined based solely on the results of poultry necropsy in the field. In the case of AI and ND disease, confirmation of laboratory diagnosis is needed, especially immunopathological immunohistochemistry as an indicator whether the chicken is infected with AIV and / or NDV in order to properly, accurately and optimally overcome it.

CONCLUSION

Based on the results of the present study it is evident, that pathological lesions (anatomical and histopathological) focal hemoragic necrosis in the digestive tract (proventriculus and small intestine) cannot be used in confirmation of the diagnosis of AIV and / or NDV because of similar. To confirm the diagnosis of AIV and / or NDV, if a gastrointestinal tissue sample is used through a pathological examination approach, an immunohistochemical immunopathological immunologic approach to streptavidin biotin and polyclonal and / or monoclonal antibodies is needed to confirm the diagnosis.

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