The Clinical Symptom and Anatomical Pathology of Tolaki Chicken which Infected Newcastle Disease Viral

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ABSTRACT

This study aim was observatiion the clinical and anatomic pathology Tolaki chicken infected virus Newcastle Disease (ND) as an affirmation analysis of the cause of the viral disease. A total of 30 Tolaki chickens divided into 3 groups based on the Mx (Myxovirus) gene genotype. Mx gene is a gene that is antiviral, producing three genotypes (AA, AG and GG), respectively 10 chicks kept in separate cages. Challenge test performed by infection with a virus gene ND VII eye drops at a dose of 104 CLD50 /0.5 ml / head. The results showed that in the first week of chicken genotype AA, AG and GG generally show the same clinical symptoms such as ducking, breakaway, face swollen, snoring. Chicken looks decreased appetite and green diarrhea are found in the feces. The number of chickens that died at the AA and AG there are three tails, while the GG Chicken found 6 tail. In the second week the AA genotype was not found green diarrhea, while the AG and GG discovered green diarrhea. The number of chickens that died at the AA and AG there are two tails, while GG was found dead three tails. Total chickens that died at the AA and AG found as many as 5 tails, while the GG as many as 9 tails. Generally the infected chicken ND virus causes swelling of the spleen, bleeding/redness of the intestine and trachea in all genotypes. Swelling of the spleen in the AA genotype was found to be 20%, AG and GG 60% of 100%. Spleen were obtained blackish color on the GG genotype of 44.44%. Based on changes in anatomic pathology in this study was obtained characteristics ND diseased chickens, so the chickens have been infected with the virus diagnosed ND. Patognomosis changes in anatomical pathology characterized by hemorhagi enteritis in the gastrointestinal tract (gut) and respiratory tract (trachea). Based on the results of the ND virus challenge test to prove the group challenged chickens have been infected with ND virus. ND virus infection attacks the intestinal organs, trachea, and spleen affecting the decline of the immune system in all genotypes. AA and AG genotypes have better protective properties against ND virus infection than genotype GG.

Key Words: Clinical Symptom, Anatomy Pathology, Tolaki Chicken, and ND Viral

INTRODUCTION

Disease resistance is a natural mechanism of each living creature in order to survive. Indicators of real resistance to the disease can be seen in adaptability and subsequently reproduce well on an environment. Local chickens are able to adapt to the poor environment, are able to live in the wild and resistant to disease. This suggests that local chickens have the ability immune response in the form of self-defense mechanism against infectious diseases including viral. This defense mechanism is controlled by a number of genes that are antiviral particularly Mx gene (Myxovirus) (Maeda, 2005; ;Pagala and Nafiu, 2012 ; Pagala et al, 2013). Chicken has a very high genetic diversity. This is one of the strong basis why genetic diversity is associated with chicken resistance against several diseases including influenza virus attacks, there is a strong correlation between high genetic diversity and frequency of alleles in the gene responsible for resistance to a disease of chicken. This

is evidenced by studies showing a high frequency distribution of the A allele (genotype AA), which is resistant to influenza viruses in chicken left alive in the wild, in contrast to the chicken broiler which has undergone a process of unification of genetic shows the distribution of frequencies of alleles A very small close to zero (Sulandari et al. 2007). Indicators of chickens to disease resistance can not only be based on genetic diversity in a group of antiviral genes, but can also be based on clinical symptoms and pathology, anatomy. This study aimed to examine the association between genotype Mx gene with the ability of immune response in chickens infected Tolaki Newcastle Disease virus

MATERIALS AND METHODS

Place and Time

The research location is housed in cages Poultry Field Laboratory Faculty of Animal Breeding Unit haluoleo university. DNA extraction and amplification of DNA Mx gene performed at the Laboratory of Molecular Breeding and Genetics Department of the Faculty of Animal Husbandry IPTP. The research was conducted from April to August 2013.

Research Material

The sample used in this study is the Tolaki chicken taken from several areas in South Konawe Southeast Sulawesi province as much as 25 tails. Blood sampling and feathers done in Phase I (first) study, further laboratory analysis at the second stage in the Laboratory of the Faculty of Animal Husbandry Department IPTP. Materials used are chemicals for blood sampling, extraction of DNA, DNA amplification, gel electrophoresis, and Silver Staining include: EDTA, Trs, HCL concentrated, potassium acetate, deionized water, NaCl, NaOH, SDA, sodium acetate, acid acetate, phenol, absolute ethanol, chloroform, isoamyl alcohol, and proteinase K, agarose, bromfenolblue, ethidium bromide, DNA marker 100 pb leader, Tris-Borate-EDTA, MgCl2, dNTP, Taq polymerase and random primers.

The tools used PCR machine, electrophoresis, autoclave, vacuum tainer, micro pipette, pipette tips, disposable glove, centrifuge, vortex, beaker, magnetic stirrer, eppendorf tube, appliance thremocycler, UV lights, camera paraloid.

Research Methods

DNA extracted from blood samples of blood samples in tubes EDTA take in to the micro tube (1.5 ml) was then added to 1000 mL of DW / TE (NaCl 0.2%). After vortex and allowed to stand 5 minutes, then centrifuged at a speed of 8000 rpm for 5 minutes. Supernatant solution formed discarded. The next phase, the addition of 40 mL of 10% SDS, 10 mL proteinase K 5 mg / ml and 1 x STE (Sodium Tris EDTA) as much as 300 mL. The next solution is gently shaken in an incubator at 55 0C for 2 hours. Phenol was then added 400 mL, 400 mL CIAA (Chloroform: Isoamyl alcohol = 24: 1), and 40 mL of NaCl 5 M shaken slowly at room temperature for 1 hour and then centrifuged at a speed of 12000 rpm for 5 minutes. Clear solution containing DNA was transferred as much as 400 mL to 1.5 mL eppendorf tube new. Then added 800 mL of EtOH (absolute ethanol) and 40 mL of NaCl 5 M then stored in the freezer overnight. The next phase solution was centrifuged again at a speed of 12000 rpm for 5 min, the supernatant was discarded and ignored formed in an open state or in a desiccator until the alcohol is gone. The last stage was added 100 mL of TE 80% or elution buffer which serves as a buffer. DNA obtained is then stored in the freezer until used (Sulandari and Zein, 2013).

DNA extracted from samples

Feather samples that can be used a whole feather sample, which has a section chalamus. DNA extraction from feather samples was performed using extraction kit Phire Animal Tissue Direct PCR Kit (Thermo Fisher Scientific Inc.). Extraction procedure carried out following the instructions of the kit manufacturer as follows: ± 0.5 cm in the first part (root / chalamus) fur transferred into 1.5 ml

tubes, then cut to be some small part. At the 1.5 ml tube was added 20 mL dilution buffer and 0.5 mL of DNA Release ™ Additive. The mixture is stirred using a vortex tube and then centrifuged. After the mixture was incubated for 2-5 minutes at room temperature and continued for 2 minutes at a temperature of 98 ° C. DNA samples ready for use or stored at -20 ° C for use later. Sambrook et al, 1989)

Segment Mx Gene Amplification by PCR

DNA samples were amplified by PCR machine (Polymerase Chain Reaction) (Muladno, 2010). Specific primers to amplify the gene Mx based Sironi et al. (2010) is the foward primer (5'-GCA TCA CCT CTG CTT AAT AGA-3 ') and reverse (5'-TTG GTA GTA GGC TTT GTT GA-3'). DNA amplification performed in a total volume of 25 mL consists of 2 mL (10-100 ng) of DNA, 15.75 mL sterile deionized water; 2.5 mL of 10 × buffer without Mg 2+; 2 mL MgCl2; 0.5 mL of 10 mM dNTP; 0.25 mL of Taq polymerase; 2 mL (25 pmol) primer. Phase 1 study with 1 x cycle, covering the initial denaturation at 94 ° C for 4 minutes. Phase II carried out with 30 x cycle, including denaturation at 94 ° C for 10 seconds, annealing at 60 ° C for 1 min, the elongation of the DNA molecule at a temperature of 72 ° C for 2 minutes. Phase III is done with 1 x cycle, covering the end of the DNA molecule elongation at 72 ° C for 7 min. Incubate at 4 ° C until used for further analysis. Electrophoresis Electrophoresis of DNA fragments amplified by PCR was performed using the device electrophoresis on 2% agarose gel (0.5 g / 25 ml of 0.5 X TBE). Road device using 0.5 X TBE buffer, at a voltage of 100 volts for 30 minutes. Viasualisasi gel elktroforesis performed on the gel documentation Alpha Imager (Alpha Imager).

Data Analysis

Based on the results of DNA extraction and amplification is then performed a qualitative analysis by comparing the DNA bands generated view of the two methods of extraction of DNA in gel electrophoresis.

RESULTS AND DISCUSSION

Symptoms Clinical and Anatomical Pathology Chicken Challenge

Early action undertaken in the handling of cases of the disease in poultry is to analyze the cause of which is the source of the disease. One approach in analyzing the cause of the disease is to look at the clinical symptoms and continued by analyzing the picture after death in the form of anatomical pathology of poultry nekropsi.

Clinical symptoms

Tolaki chickens infected with ND virus providing immunity response in the form of clinical symptoms as shown in Table 1.

Table 1. Clinical symptoms chickens challenged with the ND viral

Clinical symptoms	First Week			Second Week		
	AA	AG	GG	AA	AG	GG
Ducking	+	+	+			
Secede		+	+		+	
Decreased appetite	+			+	+	+
Face swelling	+			+	+	+
Snoring	+		+	+		+
Diarrhea Green	+	+			+	+
Exudat				+		
Mortality	3	3	6	2	2	3

Description: (+) = The clinical symptoms identified

Based on data from Table 1, shown in the first week of chicken genotype AA, AG and GG showed the same clinical symptoms such as ducking, breakaway, face swollen, snoring. Chicken looks decreased appetite and diarrhea are found in the stool green. Differences were seen in clinical symptoms evenly AA, AG predominantly on green diarrhea, while the GG dominated by ducking, splits and snoring. The number of chickens that died at the AA and AG there are three tails, while Chicken GG 6 tail was found dead. In the second week, chicken genotype AA, AG and GG still exhibit similar clinical symptoms but with greater frequency (dominant). Differences were seen in AA was not found green diarrhea, while the AG and GG discovered green diarrhea. The number of chickens that died at the AA and AG there are two tails, while GG was found dead three tails.

Based on the clinical symptoms group of chickens who are challenged, such as swelling of the face and eyes, feces green and chicken looked lethargic with a decreased appetite drastically to cause death suddenly in chickens showed chickens have been infected with ND virus belonging to the types of viscerotropic velogenic newcastle disease (VVND) (Alexander 2003; Alders and Spadbrow 2001). Based on the speed of the spread of the virus (morbidity) and the number of chickens that died (mortality) in Table 4.3, shows the first week of AA and AG genotype is almost the same with the chicken deaths each 3 tails, while the GG genotype mortality is high enough that 6 tail. Similarly, in the second week of AA and AG genotypes dtemukan dead chickens each 2 tail, while the GG was found dead chickens as many as 3 heads. Total chickens that died at the AA and AG found as many as 5 tails, while the GG as many as nine tails. This means the high pattern of morbidity and mortality caused by viral infections this ND. Newcastle disease is a disease which has been classified as a respiratory disease in chickens, which have the clinical symptoms is similar to other respiratory diseases such as AI, IB, ILT, and CRD Coryza, the difference is in the epidemiological and clinical characteristics of the particular. Newcastle disease and AI has a similar epidemiological characteristics, namely the speed of the spread of disease (morbidity) and the number of chickens that died (mortality) is very high (Tarmudji 2005).

Chickens that have resistance to the disease tends to look normal and showed no clinical signs or commonly called sub-clinical manifestations. In this study obtained challenged chickens that do not show clinical symptoms even though the virus has infected chickens ND, namely each as much as 5 mice in AA and AG genotypes. Allegedly these chickens showed symptoms of subclinical manifestations. Sub-clinical manifestations can occur when the disease in a region already is endemic. In this condition, disease agent does not cause symptoms in landlord and landlady did elimination of the agent of the disease.

In principle, the subclinical manifestations of ND, occurs when the landlady (chicken) to form an inadequate immune response, so it is not quite able to kill the infecting virus as a whole. Some causes of manifestations of subclinical ND, including: The nature of viruses are always changing so that antibodies in chickens did not recognize him perfectly, seed vaccine less proportional to the level of homology seed vaccine and the virus is very low, the application of the vaccine is not appropriate, chicken only vaccinated once so production antibodies are not adequate, and the condition of the disease was endemic. (Wibawan 2012).

Pathology (PA)

In the diagnosis of disease in chickens, in addition to general clinical symptoms may be followed by anatomical pathology approach in the form of post-death examination after surgery chickens. Table 2 shows the changes in anatomical pathology chickens infected with ND virus.

Diagnosis of the disease in animals can be done through the examination of histological, serological, bacteriological, virological and after death, but if there is a high mortality rate that can be performed surgical technique carcass examination (necropsy) to get results fast and accurate diagnosis (Tarmudji 2005). As a provisional diagnosis to prove the existence of acute Newcastle disease, it can be done based on examination of the epidemiology, clinical symptoms, and the last is the pathological changes that patognomosis before confirmation of the diagnosis made based on the results of the isolation and identification of viruses (Alexander 2001).

Table 2. The Changes of anatomic pathology groups of chickens were challenged with the ND viral

Anatomic Pathology	Chickens Challenged (Living)			Chickens Challenged (Dead)		
	AA (5)	AG (5)	GG (9)	AA (5)	AG (5)	GG (1)
Swollen spleen	1/5	3/5	9/9	0/5	0/5	0/1
Black spleen	0/5	0/5	4/9	0/5	0/5	0/1
Intestinal bleeding	5/5	5/5	9/9	0/5	0/5	0/1
Tracheal bleeding	5/5	5/5	9/9	0/5	0/5	0/1

Description: (..) = number of samples

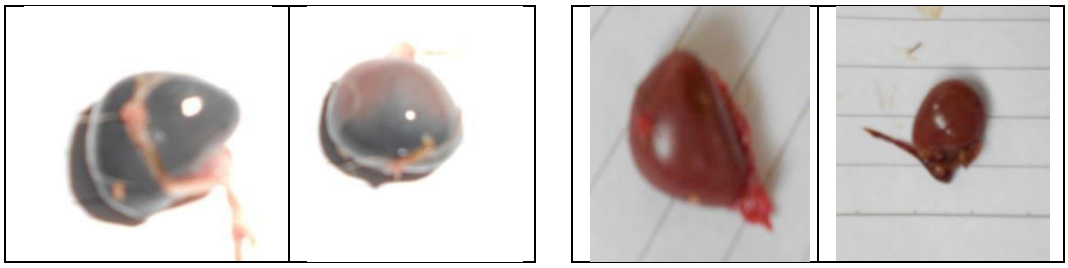
Diagnostic pathology approach requires the examination and the conclusion of an illness based on the observation of abnormalities of cells, tissues, or organs due to a disease process. Some diseases that have pathological changes that distinguish (patognomosis), diagnosis patologiknya will have a high degree of accuracy. Approximately 90% of disease in chickens are most commonly found in Indonesia can cause damage to the macroscopic and microscopic specific tissue / organ targeted, so that the disease can be diagnosed by the PA, but when changes in the PA does not distinguish, then the determination of the diagnosis should be supported by laboratory tests.

Table 3. Percentage Changes Of Anatomic Pathology Of Post Dead Chickens Were Challenged in Tolaki Chicken

Pathologi anatomi (PA) changes	Genotipe		
	AA (5)	AG (5)	GG (9)
Swelling of the spleen (%)	20.00	60.00	100.00
Spleen color black (%)	0.00 (Figure 4.1b)	0.00 (Figure 4.1b)	44.44 (Figure 4.1a)
Intestinal bleeding / hemorhagi enteritis (%)	100.00 (Figure 4.2a)	100.00 (Figure 4.2a)	100.00 (Figure 4.2a)
Tracheal bleeding / hemorhagi enteritis (%)	100.00 (Figure 4.3a)	100.00 (Figure 4.3a)	100.00 (Figure 4.3a)

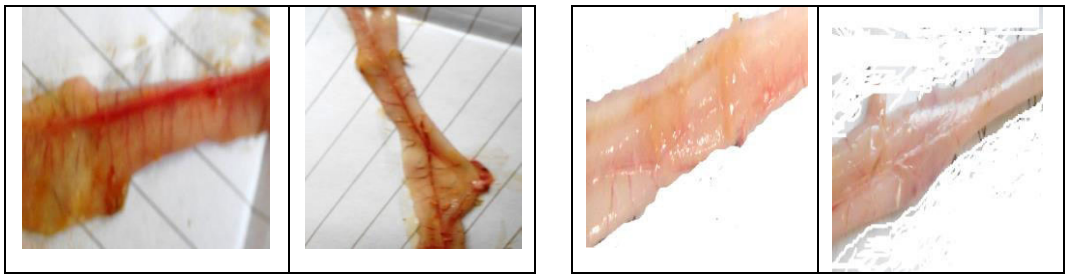
Surgery done on the carcasses of chickens that died after challenge test to see pathological anatomy. Generally the infected chicken ND virus causes swelling of the spleen, bleeding / redness of the intestine and trachea in all genotypes. Swelling of the spleen in the AA genotype was found to be 20%, AG and GG 60% of 100%. Spleen were obtained blackish color on the GG genotype of 44.44%. Based on changes in anatomic pathology in this study was obtained characteristics ND diseased chickens, so the chickens have been infected with the virus diagnosed ND. Patognomosis changes in anatomical pathology characterized by hemorhagi enteritis in the gastrointestinal tract (gut) and respiratory tract (trachea) as stated Kencana and Kardena (2011); Tabbu (2000) that changes in anatomical pathology patognomosis on Newcastle disease characterized by hemorhagi enteritis in proventriculus, ventrikulus, wipe the tonsils, colon, trachea and lungs.

Changes in anatomical pathology of chickens infected with ND virus is presented in Figure 1, Figure 2 and Figure 3



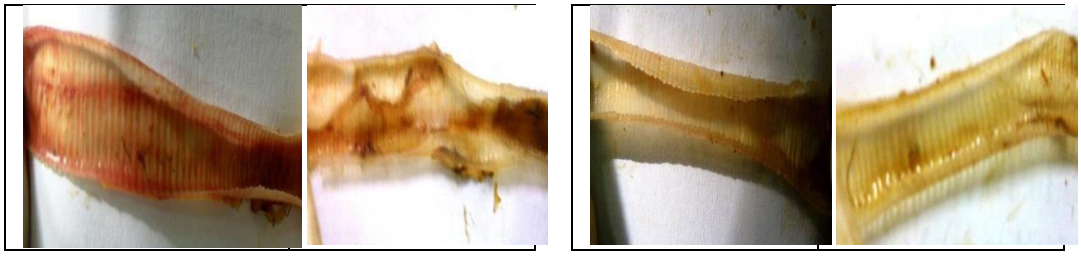
(a) (b)

Figure 1. Spleen; (a) = black spleen organ found in chickens challenged GG after death; (b) = spleen is normally found in chickens challenged AA and AG were still alive as well as the control chickens.



(a) (b)

Figure 2. Intestinal organs; (a) = bleeding intestinal organ found in all genotypes after dead chickens challenged; (b) = normal intestinal organ found in chickens challenged surviving and control chickens



(a) (b)

Figure 3. Organ trachea; (a) = bleeding tracheal organ found in all genotypes after dead chickens challenged; (b) = normal tracheal organ found in chickens challenged surviving and control chickens

Anatomical pathology seen in the form of swelling of the spleen and blackish in color (only in genotype GG), bleeding in the trachea and intestine at all chickens challenged indicated ND virus has spread to all organs and managed to penetrate the mucosa propria. ND virus infection were given lower the durability of chicken. ND pathogenic viruses causing damage to several organs and the impact on mortality of chicken (Alexander 2001)

CONCLUSION

Based on the results of the ND virus challenge test and fenotiping in this study, proving the group challenged chickens have been infected with ND virus. ND virus infection attacks the intestinal organs, trachea, and spleen affecting weight gain decline in all genotypes, whereas the control group showed weight gain chicken better at AA and AG genotypes compared to genotype GG. AA and AG genotypes have better protective properties against ND virus infection than genotype GG.

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