

Association of Mx Gene Genotype with Antiviral and Production Traits in Tolaki Chicken

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Abstract: Tolaki chicken is a kind of Indonesian local chickens, that belong to the ability of anti viral responses. This ability is controlled by the present of antiviral Mx (myxovirus resistance) gene. The Mx gene codes for a protein with antiviral activity. The objective of the study was to prove the tolaki chicken Mx gene genotype is associated with antiviral and production traits. Mx/Hpy 81 gene was genotyped in 103 tolaki chickens with PCR-RFLP. A total of 30 chickens were challenged with ND gen VIIb virus (10^4 CLD₅₀)/chicken. PCR was used to amplify genomic DNA for Mx gene (299 bp). The amplimer was cut by Hpy 81 produce three genotypes: AA, AG and GG and two alleles: A allele (299 bp) and G allele (200 bp and 99 bp). Frequency of A allele (0.74) was higher than G allele (0.26). The all parameters of production traits in challenge test group were not significantly different in AA, AG and GG genotypes. The daily weight gain, feed intake and FCR were significantly different in AA, AG and GG genotypes of chickens control group. The parameters of antiviral traits showed that vitality of AA (50%) and AG (50%) of chickens were better then GG (10%) in challenge group. The vitality of AA (100%) and AG (100%) were better GG (33.33%) in control group. The study postulated that Mx gene genotype could be associated with production and antiviral traits in tolaki chicken. AA and AG genotype are more resistant and show better production than GG genotype.

Key words: Tolaki chicken, newcastle disease, genotyping and Mx gene

INTRODUCTION

Newcastle Disease is one of the disease that often strikes in the poultry industry. Newcastle Disease is a contagious disease that attacks chickens at various ages with high mortality rates (Alexander, 2001). This disease can spread through mucus, feces, direct contact with sick chickens, dust, equipment enclosures, chaff or wind, insects and wild birds. The clinical symptoms is begun by the present of respiratory interference, beak open, coughing, sneezing, snoring sound. The appetite of chicken was decreased and it was greenish watery stool. Furthermore the symptoms was followed by neurological symptoms such as muscle trembling, walk backwards, down and rotate the head up and followed by paralysis (Zainuddin and Wibawan, 2007).

Local chickens has a high genetics diversity. This fact is a basic reason that the chicken can adapt in the bad environment, spread out in wild life of nature and resist to several diseases, such as *Avian Influenza* (AI) and Newcastle Disease (ND). The resistance of the virus infections is controlled by antiviral genes (Sartika *et al.*, 2010).

Tolaki chicken is one of the local chicken from South East Sulawesi (Sarwono, 2005). The color patterns of tolaki chicken have similar with the red jungle fowl (*Gallus gallus*). It is one of the 31 kinds of local chickens that have characteristic typical appearance (Nataamidjaja and Dwiyanto, 1994). Pagala and Nafiu (2012) reported that a posture of tolaki chicken have relatively smaller and slimmer body shape than kampung chicken. The average weight of cocks are 1.60 ± 0.29 kg and hens are 1.29 ± 0.21 kg. Most of the tolaki chickens were traditionally used by Tolaki's people as a media of ritual and traditional medicine, tolaki chicken is believed immune to some diseases (Pagala and Aku, 2010).

Naturally, native chickens has ability to respond the virus controlled by several antiviral genes. Mx gene has been found to be specific genes that control the ability of the chicken became resistant or sensitive to virus attacks. Mx gene can lead to resistance to vascular stomatitis virus (VSV) and Avian Influenza (AI) (Ko *et al.*, 2002; Maeda, 2005). Mx gene produces Mx protein. The Mx proteins are key components and its coding protein had been shown to be induced by interferon (IFN) and to inhibit the replication of RNA virus.

Watanabe (2003) studied chicken Mx cDNAs from other breeds to see whether these chickens carried resistant or sensitive character of the Mx gene to the VSV/vesicular stomatitis virus infection. Only an amino acid substitution at position 631 was identified to determine the difference between the antiviral activity of chicken Mx protein; Asparagine (Asn) corresponded to the positively antiviral activity and serine (ser) corresponded to the negatively antiviral activity Sulandari *et al.* (2007) stated that most of the local chicken population in Indonesia showed the Mx gene polymorphism at nucleotide position 1892 in exon 13. Mx gene polymorphism genotype is determined by the presence of the G allele (GG genotype) that are sensitive to virus attacks AI/ND, the A allele (genotype AA) are resistant to virus attacks AI/ND. The G/A polymorphism at position 2032 of chicken Mx cDNA resulting in the substitution of serine with asparagine at position 631 of the Mx protein, seems to influence the antiviral activity of the molecule (Ko *et al.*, 2002).

The resistance of native chicken was correlated to the performance activity. Generally, the animal that have a high fitness level and able to respond a disease infection, it will be shown better production.

The objective of the study was to prove the association between polymorphisms genotype of Mx gene tolaki chicken with production and antiviral traits.

MATERIALS AND METHODS

The research was conducted during six month (April to September, 2013), at Laboratory of Poultry Breeding Faculty of Animal Science Halu Oleo University Kendari, Molecular of Animal Breeding and Genetics Laboratory Faculty of Animal Science, Bogor Agricultural University, Bogor.

Animal experiments and rearing: A total 103 tolaki chickens DOCs were reared intensively for 8 weeks. The tolaki chickens were randomly placed at cages. The cage size was 60x60x40 cm³. All of the cages placed in a pen (5x15 m²). The feed was commercial feed for growing hens that contain 18-19% crude protein and 2850 kcal metabolizable energy. Feed and water were given *ad libitum*.

Mx gene genotyping: A total of 103 samples of tolaki chicken was genotyped for DNA extraction. Blood sample was taken from the brachial vein in the wing area. Extraction of DNA used phenol-chloroform method (Sambrook *et al.*, 1989). DNA has been extracted in the PCR amplification. Specific primers were used to amplify the Mx gene with a forward primer (5'-GCA CTG AAT TCA AGA CTT CCT-3') and reverse primer (5'-GTA TTG GTA GGC TTT GTT GA-3'). RFLP method was used to determine the genotype Mx gene. PCR product of Mx gene fragments cut by Hpy 81 restriction enzymes that

cut the 631 sites (Sironi *et al.*, 2010). PCR products were separated by electrophoresis 2% agarose gel. Based on genotyping results calculated frequency of genotype Mx gene (Nei, 1987).

Challenge test: A total of 30 tolaki chickens were divided into 3 groups in separate cages (AA, AG and GG genotypes). Challenge test were done by contact 5 chickens infected with Newcastle Disease virus VIIb gene in eye drops (a dose of 10⁴ CLD₅₀/0.5 mL/chick). All chickens that will be tested in a mixed challenge isolator cages ND virus transmission to occur. Observations were made in the morning and evening all sick and dead chickens were recorded (Darminto, 1995).

Data analysis: Association of genotype with the observed variables were analyzed with ANOVA using completely randomized design. Mx gene genotype was as treatment and production or antiviral variables data were as response. The difference of the genotype of each gene compared using Tukey's test at 5% level. Statistical models was used: $Y_{ij} = \mu + \pi_i + \tau_{ij}$ (Matjik and Sumertajaya, 2002)

RESULTS AND DISCUSSION

Polymorphism Mx/Hpy 81 gene genotype in tolaki chickens: Mx/Hpy 81 gene was genotyped on exon 13 (Base to 2032 cDNA), with a 299 bp PCR product. The results of this study was presented in Fig. 1.

Mx gene in chickens, located on chromosome 1. Based on data from GenBank (DQ788615), the size of Mx gene was 21084 bp. The structure of the gene was preceded by a promoter region (215 bp), exon 13 in the coding region (2118 bp), 5'UTR region (140 bp) and the last is the 3'UTR region (288 bp).

Figure 1 was the result of PCR-RFLP of the Mx gene fragments (299 bp), which was cut by restriction enzymes Hpy 81, in exon 13 site to 2032 (GTN | NAC). The cutting with Hpy 81 enzyme produces two alleles (A and G) and three genotypes (AA, AG and GG). A allele can not be cut by Hpy 81 (a fragment size 299 bp), whereas the G allele may be cut by Hpy 81 (Two fragments size 200 bp and 99 bp).

Results of cutting on site to 2032, detected the presence of a base transition mutations (point mutation). This mutation caused base change from amino acid serine (AGT) to asparagine (AAT). The presence of the amino acid asparagine (A) at nucleotide number 2032 exon 13, an indicator of chicken was resistant viral infection, which was classified as Mx⁺ gene, when mutations into the amino acid serine (G) caused the chicken was sensitive to virus attacks, classified as Mx⁻ gene (Watanabe, 2003; Ko *et al.*, 2002).

Frequency of Mx/Hpy 81 gene genotype in tolaki chicken: From 103 blood samples of tolaki chicken that

was genotyped, has been produced AA genotype (63), AG genotype (27) and GG genotype (13). Genotype frequency of Mx gene of Tolaki chicken was presented in Table 1.

The frequency of AA genotype (61.17%) dominated the Tolaki chicken samples, followed AG genotype (26.21%) and GG genotype (12.62%). Tolaki Chicken has been produced the frequency of A (0.74) in the Mx gene which was higher than frequency G allele (0.26). These results was similar to previous research conducted by Maeda (2005) who reported that all populations of local chickens in ASEAN countries had been Mx⁺ gene (A allele which resistant Avian Influenza virus) and Mx⁻ gene (G allele which sensitive Avian Influenza virus), it is found in Indonesia, the frequency of Mx⁺ gene was greater (63%) and remaining Mx⁻ gene (37%). These results were similar with the results of the analysis of the distribution of A/G SNP Mx gene from Indonesian local chicken population that has been done by Sulandari *et al.* (2007) who obtained the frequency of A allele which was resistant to AI/ND ranges from 0.35-0.89.

Association of Mx/Hpy 81 gene genotype with production trait and resistance against newcastle disease virus in tolaki chicken: Association of Mx/Hpy 81 gene genotype with production trait and viral disease resistance of Tolaki chicken was presented in Table 2.

Production trait of tolaki chicken: Observations on the challenge test group showed a decrease in the daily weight gain and the increase feed intake of all genotypes, whereas the results of analysis of variance showed that the daily weight gain, feed intake and feed conversion of all genotypes were not significantly different ($p > 0.05$).

Results indicated that infected ND virus can affect the production of chickens. The high pathogen ND viruses caused damage to several organs that was disrupted the function of the organ system. Thus it can be caused the metabolism of chicken was not optimal (Alexander, 2003).

The high feed intake and low daily weight gain in all genotypes in chicken challenge test group indicated the amount of metabolic energy requirement for the survival of chicken against ND virus. Several previous studies reported that there were a tendency of animals to change their production in order to maintain the adaptability with environment including self-defense mechanism against disease.

The control group showed the daily weight gain and feed intake of AA and AG genotypes was significantly higher with GG genotypes ($p < 0.05$). These results reinforce previous initial suspicion that there was correlation between the ability of disease resistance in animal showed production (Fulton *et al.*, 2006; Otim, 2005). An animal that have a high fitness level and able to respond a disease infection, it will be shown better production.

Antiviral trait of tolaki chicken: Table 2 showed that the genotype AA and AG genotypes in challenge test groups was better resistant than GG genotypes. This indicator can be seen from the percentage of live (vitality) chicken challenged group. The vitality was 50% in AA and AG genotype and only 10% in GG genotype.

Vitality of the chicken challenge was little different from chicken control group. The percentage of the control chicken vitality AA and AG genotype was 100%, while only 33.33% in GG genotype.

Table 1: Frequency of genotype Mx gene in tolaki chickens

Samples	N	Frequency of genotype			Frequency of allele	
		AA	AG	GG	F(A/Mx ⁺)	F(G/Mx ⁻)
Tolaki chicken	103	63	27	13	0.74	0.26

Table 2: Association of Mx/Hpy 81 gene genotype with production trait and viral disease resistance of tolaki chicken

Observation of traits	Genotype		
	AA	AG	GG
Challenge test group			
The number of live chickens	5/10	5/10	1/10
Vitality (%)	50	50	10
Daily weight gain (g/chicken/day)	-2.99±0.77 ^a	-2.63±2.22 ^a	-2.12±2.02 ^a
Feed intake (g/chicken/day)	22.28±0.08 ^a	22.50±0.39 ^a	23.05±1.68 ^a
FCR	8.04±2.84 ^a	11.00±8.15 ^a	14.06±2.62 ^a
Control group			
The number of live chickens	5/5	5/5	1/3
Vitality (%)	100	100	33.33
Daily weight gain (g/chicken/day)	4.13±1.91 ^a	2.11±1.19 ^a	1.05±0.08 ^b
Feed intake (g/chicken/day)	20.77±0.03 ^a	18.04±0.02 ^a	16.92±1.74 ^b
FCR	5.77±2.05 ^a	10.10±3.63 ^b	16.18±1.56 ^b

Different superscript letters in the same row indicate significant differences ($p < 0.05$)

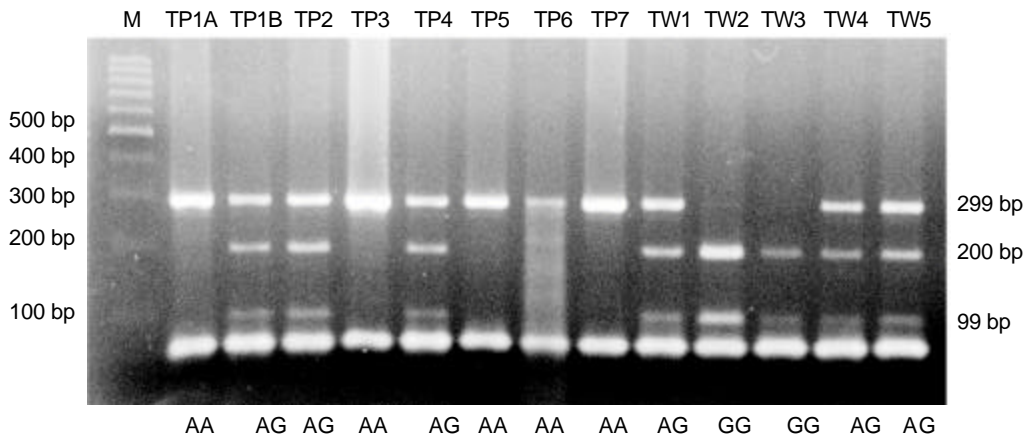


Fig. 1: PCR-RFLP amplification product of Mx gene at exon 13 was cut by Hpy 81

The high resistance of the AA and AG genotypes was caused by the presence of the A allele which contributed resistance to ND virus attacks, whereas the low resistance of GG genotype was caused by G allele which sensitive to ND virus attacks (Sulandari *et al.*, 2007). The G/A polymorphism at position 2032 of chicken Mx cDNA resulting in the substitution of serine with asparagine at position 631 of the Mx protein, seems to influence the antiviral activity of the molecule (Ko *et al.*, 2002). Mx gene produced a protein that was part of the innate immune system because of its ability to produce interferon (IFN). The production of interferon (IFN) was triggered when the viral RNA was detected and recognized by the host cell receptor. IFN induced the expression of more than 300 Interferon-Stimulated Genes (ISGs). ISGs was very effective against the virus as has a high antiviral activity such as blocking protein synthesis, degrade RNA genome and eliminate viral components directly in the location where the virus replicated (Sartika *et al.*, 2010; Pavlovic *et al.*, 1992; Matzinger *et al.*, 2013).

Conclusion: Results of genotyping Mx/Hpy 81 gene at nucleotide 2032 to exon 13 and ND virus challenge test VIIb gene (10^4 CLD₅₀ dose) was presented in this study. The data confirm that Mx gene genotype could be associated with production and antiviral traits in tolaki chicken. AA and AG genotype are more resistant against ND virus and show better production than GG genotype.

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REFERENCES

- Alexander, D.J., 2001. Newcastle Disease. *Poult. Sci.*, 42: 5-22.
- Alexander, D.J., 2003. Newcastle Disease, other Avian Paramyxovirus and Pneumonivirus Infections, p: 63-81. In: YM Saif, Barnes HJ, Glisson JR, Fadly AM, McDougald LR, Swayne, DE. 2003. *Diseases Poultry 11th Ed.* Iowa (US): Iowa Blackwell Publishing.
- Darminto, 1995. Vaksinasi Penyakit tetelo secara Kontak pada Ayam Buras: Perbandingan Analisis antara Kondisi Laboratorium dan Lapangan. *JITV.*, 1: 178-184.
- Fulton, J., H.R. Juul-Madsen, C.M. Ashwell, A.A. McCarron, J.A. Arthur, N.P. O'Shullivan and R. Taylor, 2006. Molecular Genotype Identification of The *Gallus gallus* Major Histocompatibility Complex. *Immunogenet.*, 58: 407-421.
- Ko, J.H., H.K. Jin, A. Asano, A. Takada, A. Ninomiya, H. Kida, Hokiyama, M. Ohara, Tsuzuki, Nishibori, Mizutani and T. Watanabe, 2002. Polymorphisms and the differential antiviral activity of the chicken Mx gene. *Genome Res.*, 12: 595-601.
- Maeda, 2005. Polymorphism of Mx Gene in Asian Indigenous chicken population. Seminar Nasional Tentang Unggas Lokal III: 2005 Agustus 25; Semarang, Indonesia. Semarang. Universitas Diponegoro.
- Matjik, A.A. and M. Sumertajaya, 2002. Perancangan Percobaan dengan Aplikasi SAS dan Minitab. Ed. ke-2. Bogor. IPB Press.
- Matzinger, S.R., T.D. Carol, J.C. Dutra, Z.M. Ma and C.J. Miller, 2013. Myxovirus resistance gen A (MxA) Expression supresses influenza A virus replication in alpha interferon treated primate cell. *J. Virol.*, 87: 1150.

- Nataamidjaja, A.G. and Diwyanto, 1994. Konservasi Ayam Buras Langka. Koleksi dan Karakterisasi Plasma Nutfah Pertanian. Review Hasil dan Program Penelitian Plasma Nutfah Pertanian. Bogor. 1994 Juli 26-27; Bogor, Indonesia Bogor. Balitbang Pertanian.
- Nei, M., 1987. Molecular Evolutionary Genetics. New York, Columbia University Press.
- Otim, M.O., 2005. Newcastle Disease in village poultry: Molecular and phylogenetic studies of the virus and disease epidemiology. Disertasi. Copenhagen, The Royal Veterinary and Agricultural University.
- Pagala, M.A. and A.S. Aku, 2010. Studi Sebaran dan Pemetaan Populasi Ayam Tolaki (Manu ndolaki) di Kabupaten Kolaka dan Kolaka Utara dalam rangka pelestarian plasma nutfah asli Sulawesi Tenggara. *Warta Wiptek*, 18: 151-155.
- Pagala, M.A. and L.O. Nafiu, 2012. Identifikasi molekuler sifat anti viral ayam Tolaki melalui deteksi gen Mx sebagai marka genetik. *J. Agri.*, 23: 139-144.
- Pavlovic, J., O. Haller and P. Staeheli, 1992. Human and Mouse Mx protein inhibit different step of the influenza virus multiplication cycle. *J. Virol.*, 66: 2564-2569.
- Sambrook, J., E.F. Fritsch and T. Maniatis, 1989. Molecular Cloning. A Laboratory Manual 2nd Ed. USA. Cold Spring Harbor Laboratory Press.
- Sartika, T., S. Sulandari and M. Zein, 2010. Selection of Mx gene genotype as genetic marker for Avian Influenza resistance in Indonesian native chicken. *Biomed. Cent. Proceed.*, 5: 37.
- Sarwono, B., 2005. Ayam Aduan. Penebar Swadaya. Jakarta. Hal, 16-18.
- Sironi, L., P. Ramelli, J.L. Williams and P. Mariani, 2010. PCR-RFLP Genotyping Protocol for Chicken Mx Gene G/A Polymorphism associated with the S631N Mutation. *Genet. Mol. Res.*, 9: 1104-1108.
- Sulandari, S., M.S.A. Zein, S. Paryanti, T. Sartika, J.H.P. Sidadolog, M. Astuti, T. Widjastuti, E. Sudjana, S. Darana, I. Setiawan, D. Garnida, S. Iskandar, D. Zainuddin, T. Herawati and I.W.T. Wibawan, 2007. Keanekaragaman Sumber Daya Hayati Ayam Lokal Indonesia; Manfaat dan Potensi. Buku. Edisi Pertama. Puslit Biologi, LIPI. 212 Halaman.
- Watanabe, T., 2003. Genomic analysis of antiviral resistant Mx gene in the chicken. *Lab. Animal Breeding and Reproduction*, Hokkaido University. Sapporo, Japan. International workshop on Animal Genome Analysis, KKR 2003 Nop 6; Sapporo. Hotel Tokyo.
- Zainuddin, D. and I.W.T. Wibawan, 2007. Biosekuriti dan Manajemen Penanganan Penyakit Ayam Lokal. Di dalam: Diwyanto, Prijono SN, Editor. Keanekaragaman Sumber Daya Hayati Ayam Lokal Indonesia: Manfaat dan Potensi. Bogor. LIPI Press.