p-ISSN 1410-5659 e-ISSN 2621-5144
Published by:
Unpad Press

Jurnal Ilmu Ternak Universitas Padjadjaran, March 2026, 26(1):65-71
DOI: 10.24198/jit.v26i1.66625
Available online at http://journal.unpad.ac.id/jitunpad

POLYMORPHISM OF THE MX GENE IN RIAU KAMPUNG CHICKENS

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Abstract

One of the genetic markers for avian influenza is the Mx gene. The purpose of this study was to determine the polymorphisms of the Mx gene in kampung chickens in Riau Province. A total of 61 samples from three subpopulations have been genotyped using PCR-RFLP. Allele and genotype frequencies have been calculated using PopGene. The Mx gene was amplified, yielding a 299-base-pair DNA fragment. This fragment was digested with the Hpy81 enzyme, which yielded three genotypes: the AA genotype showed by a single 299 bp band; AG genotype showed by 299, 200, and 99 bp bands; and GG genotype showed by 200 and 99 bp bands. AA genotype frequency was higher than that of AG and GG in Indragiri Hulu and Kampar. The A allele frequency was higher than the G allele frequency in two populations, i.e., Kampar at 78% and Indragiri Hulu at 70%. Kuantan Singingi had the highest heterozygosity observation value. This study concluded that the Mx gene in Kampung chickens from Riau Province was polymorphic. There were three genotypes of the Mx │Hpy81 gene, which were AA, AG, and GG, and two alleles, which were A and G. The observed heterozygosity value showed that the genetic diversity in Kampar and Indragiri Hulu subpopulation was low.

Keywords: kampung chicken, Mx │Hpy81 gene, PCR-RFLP

INTRODUCTION

Riau Province is a high-risk area for the spread of poultry diseases. It is because Riau Province is a major livestock transport route in Sumatra. One disease that continues to threaten the death rate in poultry is a disease caused by ND and AI viruses. Media Center Riau (2023) reported that more than 100 poultry in Kabupaten Kampar were confirmed positive for Avian Influenza (H5N1),). As of 2025, approximately 200 cases are still reported in Riau Province, indicating the continued presence of the disease in poultry (Kementerian Pertanian, 2025). Genetically, resistance to viruses, including the ND virus, is controlled by the Mx gene. Based on data from Gen Bank (accession DQ788615), it is located on chromosome 1 and encodes the Mx protein, which functions as a promoter of resistance to viral infections. The Mx gene has reportedly been associated with resistance in chickens to viral infections, particularly Avian Influenza (AI) and Newcastle Disease (ND).

In Indonesia, there are various types of local chickens, both original and adapted, which have existed for tens or even hundreds of years. Local chickens that lack unique characteristics are called Kampung chickens Tribudi et al., (2025). Kampung chickens have many advantages: they can survive and breed well despite low-quality feed, are highly adaptable to harsh environmental conditions, exhibit strong disease tolerance, have good reproductive performance under minimal management, and are suitable for scavenging production systems (Fitra *et al.,* 2021). Generally, kampung chicken maintenance is with an extensive system. So, there is a potential for infection with the virus.

Identifying polymorphism in the Mx gene is important because it helps to determine a chicken's resistance to viruses. Polymorphism

Submitted 07 September 2025; accepted 26 February 2026.
Available online 31 March 2026.

of the Mx Gene in KUB Chicken has been reported by Sartika *et al.* (2021). The result showed that the frequency of A allele was greater than G. Another studies using the PCR-RFLP method have shown that the Mx+ (AA) gene frequency of indigenous chickens in Bangladesh was 0.48, so they were more resistant to AI disease (Alam et al., 2022). Sartika *et al.,* (2021) reported allele Asn631 was resistant to the highly pathogenic H5N1 avian influenza (HPAI) virus, because produces a functional antiviral Mx protein that inhibits influenza virus replication. This research aims to identify polymorphisms in the Riau population of kampung chicken using the PCR-RFLP Method.

MATERIALS AND METHODS

Blood Samples

The total number of blood samples used in this research was 61. They were collected from three subpopulations: Kampar (n=30), Kuantan Singingi (n=21), and Indragiri Hulu (n=10). DNA was extracted using the Genaid DNA mini kit. Blood samples for DNA extraction were collected by a qualified veterinarian.

Primer

A pair of primers was used in amplification. The method is Polymerase Chain Reaction (PCR). The nucleotide sequences of the forward and reverse primers are shown in Table 1 (Alam et al., 2022). (Sironi *et al.,* 2010).

DNA Extraction

DNA extracted using the Geneaid Extraction Kit. Genomic DNA was extracted from whole blood using a spin-column protocol consisting of sample preparation, cell lysis, DNA binding, washing, and elution steps. During sample preparation, 300 µL of blood was mixed with RBC Lysis Buffer, incubated at room temperature for 10 min, and centrifuged at 3,000 × g for 5 min to obtain the leukocyte pellet. For cell lysis, the pellet was treated with 200 µL GB Buffer, vortexed, and incubated at 60 °C for at least 10 min. DNA binding was achieved by adding 200 µL of absolute ethanol to the lysate, then transferring the mixture to a GD spin column and centrifuging at 10,000 × g for 1 min. The column was washed sequentially with W1 Buffer and Wash Buffer and centrifuged to dry the membrane. Finally, DNA was eluted by applying 100 µL preheated Elution Buffer (or TE buffer/nuclease-free water) to the column membrane, incubating for 3–5 min, and centrifuging at 10,000 × g for 1 min (Putra *et al.,* 2021).

DNA Amplification

DNA was amplified using the PCR method, with a total volume of 25 µL, consisting of DNA template 2 µL, primer (forward and reverse) 1 µL, Taq polymerase 1 µL, buffer 1.5 µL, MgCl 0,5 µL, and distilled water to reach the final volume. PCR was conducted using the GeneAmp® PCR System 9700 (Applied Biosystems™), starting with a pre-denaturation step at 94 °C for 5 min. The reaction then proceeded through 35 cycles, consisting of denaturation (94 °C for 45 s), annealing (62 °C for 45 s), and extension (72 °C for 1 min), the last step is a final extension at 72 °C for 5 min (Afifah *et al.,* 2020).

Genotyping (RFLP Analysis)

RFLP analysis was employed for genotyping. PCR products (5 µL) were digested with 2 µL the restriction mix (1µL of dH2O, 0.7 µL of buffer, and 0.3 µL of cutting enzyme), the Mix incubated for 16 hours at 37°C, and the resulting fragments were separated on a 2% agarose gel in 0.5 × TBE buffer at 100 V for 40 min, followed by visualization using an Alpha Imager System (Pagala *et al.,* 2017).

Data Analysis

Genotype frequency is the proportion of a specific genotype within a population. Allele frequency is the proportion of a particular allele relative to all alleles at a given locus in the population. The mathematical models for calculating genotype and allele frequencies (Nei & Kumar, 2000) are as follows:

Genotype Frequency:

$$Xii = \frac{nii}{N} X\ 100\%$$

Allele Frequency:

$$Xi = \frac{2nii + nij}{2N}$$

Xii indicates the frequency of individuals with the *ii* genotype, *nii* represents the number of

individuals observed with the *ii* genotype, while *nij* denotes the number of individuals exhibiting the *ij* genotype. *N* is the total number of sampled individuals in the population. The degree of heterozygosity, both observed (*Ho*) and expected (*He*) were calculated with the formula as follows:

$$Ho = \sum_{i \neq j} \frac{N1ij}{N}$$

$$He = 1 - \sum_{i=1}^{q} Xi^2$$

Where *Ho*: the heterozygosity observation value, *N*1*ij*: the number of individuals with heterozygous, *N*: the observed number of individuals, *He*: the heterozygosity expectation value, *xi*: the frequency of allele, *q*: the number of alleles.

RESULTS AND DISCUSSION

The Mx gene was successfully amplified at 62 °C. These results were visualized on an agarose gel, as shown in Figure 1. The amplified product was 299 bp. In line with previous research, the Mx gene was 299 bp (Permatasari *et al.,* 2015; Sartika *et al.,* 2021; Alam *et al.,* 2022). The Mx Gene in chickens is located on chromosome 1. The gene structure included promoter, 13 exon as coding region, 5'UTR region, and 3'UTR region. Exon 13 was frequently reported to be associated with Avian Influenza resistance.

Determination of Mx Genetic Genotype with RFLP

Based on the PCR-RFLP method using the Hpy8I enzyme, three types of kampung chicken Mx gene genotypes related to the resistance to avian influenza. Namely, AA, AG, and GG (Figure 2) were assigned the AA or Mx++ genotype if there was one DNA fragment (band) of 299 bp; the AG or Mx+- genotype is indicated by three DNA fragments, namely 299, 200 and 99 bp; the GG or Mx-- genotype is indicated by two DNA fragments, namely 200 and 99 bp (Alam et al., 2022).

Based on the amplified DNA sequence in the Mx gene segment, one Hpy8I cutting site produces fragments of 200 and 99 bp. A previous study on broiler chickens (White Leghorn) reported that the diversity of the Mx gene was caused by a mutation in the exon 13 region (g.631G>A) (GenBank accession number DQ788615). The mutation in the Mx gene fragment Hpy8I was a single-base transition (single mutation), specifically G to A (purine to purine), which changes the amino acid serine (AGT) to asparagine (AAT). The amino acid asparagine at nucleotide 631 of exon 13 indicates that the chicken was resistant to AI, as indicated by the Mx++ (AA) gene. If the mutation is a base mutation at the serine codon, then the chicken is susceptible to bird flu, as indicated by the Mx--(GG) gene (Sartika *et al.* 2021).

Genetic Diversity of Mx Genes in Kampung chickens

Genetic diversity was assessed using genotypes and allele frequencies calculated from the number of individuals per genotype (Table 2). The genotypes and allele frequencies of the Mx|Hpy8I gene of Riau Province Kampung chickens are shown in Table 3.

Table 3 shows that the AA genotype frequency was higher than the GG genotype in Kampar and Indragiri Hulu. However, in the Kuantan Singingi sub population, the AG genotype was higher than the AA and GG genotypes. In this population, Kampung chicken has developed an intensive system, but in Kampar and Indragiri Hulu, it has developed an extensive system. Environmental conditions could indirectly influence genotype frequencies through natural selection, as different genotypes vary in fitness across environments. This phenomenon is related to the genotype-by-environment (G×E) interaction, in which genotypes respond differently to environmental conditions, leading to differences in livestock performance across environments (Erdem & Savaş, 2021).

Genotype frequencies are in line with the allele frequencies. A G allele frequency was lower than that of the A allele in Kampar and Indragiri Hulu. The total data also showed that the G allele frequency was lower than the A allele. This result was in line with the previous study. The frequency of the G allele was lower than that of A in several local chicken breeds in Indonesia, they were Tolaki Chicken from Sulawesi Island and Kampung chickens from Kendari City of Southeast Sulawesi Province (Pagala *et al*., 2017).

Allele frequency is the relative frequency of an allele or the total number of alleles found in a population (Rahmawati et.al., 2023). The frequency of the A allele was higher in Kampar

and Indragiri Hulu, and the frequency of the G allele was higher in Kuantan Singingi. According to Angst (2024), allele and genotype frequencies between subpopulations can determine genetic diversity between subpopulations. The GG genotype occurred at low frequency in two populations, which may indicate negative selection against it due to lower adaptive or productive performance compared with alternative genotypes, as well as non-random mating (Fathi et al., 2018).

Genotype diversity can be measured by heterozygosity (Ho); a value below 0.5 indicates low diversity in the population (Rani *et al*., 2023). The present study showed that the expected heterozygosity was higher than the observed heterozygosity in the Kampar and Indragiri Hulu subpopulations (Table 4).

Table 1. Mx │Hpy81gene

Primer	Base	TM	PCR Product
Forward	5'-GCACTGTCACCTCTTAATAGA-3'	62°C	299 pb
Reverse	5' - GTATTGGTAGGCTTTGTTGA-3'.		

Table 2. Results of genotype identification of Mx genes in Riau Kampung chicken

No	Sub Population	n	Genotipe		
			AA	AG	GG
1	Kampar	30	18	11	1
2	Indragiri Hulu	10	5	4	1
3	Kuantan Singingi	21	3	12	6
	Total	61	26	27	8

Table 3. Genotype and Allele Frequency Values of Mx|Hpy8I Gene in Riau Kampung Chickens

No	Sub Population	N	Frequency of Genotipe			Frequency of Alele	
			AA	AG	GG	A	G
1	Kampar	30	0,60	0,36	0,03	0,78	0,22
2	Indragiri Hulu	10	0,50	0,40	0,10	0,70	0,30
3	Kuantan Singingi	21	0,14	0,57	0,29	0,43	0,57
	Total	61	0,43	0,44	0,13	0,64	0,36

Table 4. Heterozygosity Observed (Ho), Heterozygosity Expected (He), and Chi Square value of Mx gene in Riau Kampung chicken

No	Sub Population	N	Ho	He
1	Kampar	30	0,36	0,65
2	Indragiri Hulu	10	0,40	0,56
3	Kuantan Singingi	21	0,52	0,50

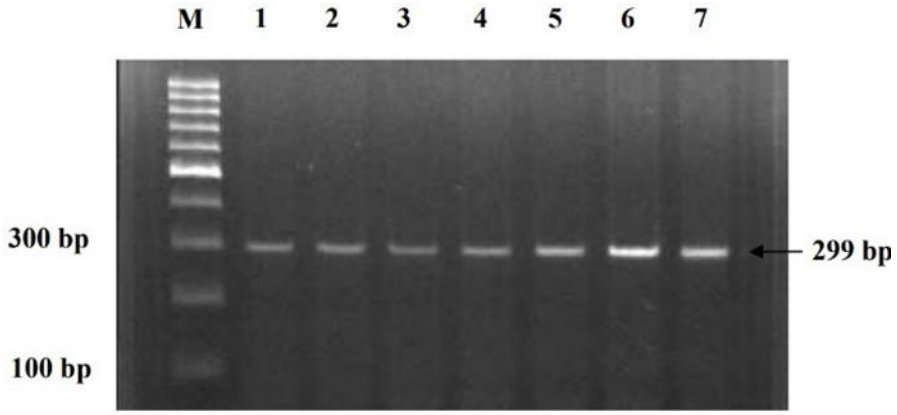

Figure 1. PCR amplification of chicken Mx gene (bp=base pair)

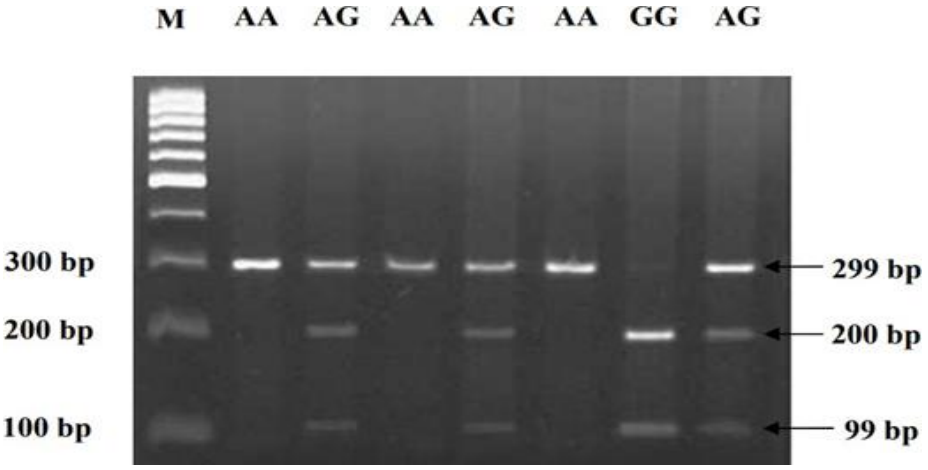

Figure 2. Banding pattern of Mx gene in Kampung chicken in 2% agarose gel with Hpy8I restriction enzyme
(M: Marker 100 bp, AA, AG, and GG are genotypes of Mx|Hpy8I gene).

An expected heterozygosity (He) value exceeding the observed heterozygosity can indicate local inbreeding in the population (Schmidt *et al.,* 2021). When observed heterozygosity is higher than expected heterozygosity, it indicates random mating in the population (Chesnokov & Artemyeva, 2015). The heterozygosity value is important to know because it can describe a population's genetic diversity. Several factors influenced this value, including the number of alleles, the allele frequency, and the number of samples (Salsabila *et al.,* 2022). This pattern is consistent with findings in Indonesian local chickens reported by Sartika *et al.* (2023), who also observed that expected heterozygosity exceeded observed heterozygosity (He > Ho), indicating a deficit of heterozygotes in the population.

CONCLUSIONS

Based on the research, the Mx gene in native chickens in Riau Province was polymorphic. There were three genotypes: AA, AG, and GG, and two alleles: A and G. The observed heterozygosity value showed low genetic diversity in the Kampar and Indragiri Hulu subpopulations. The development of native chickens carrying the AA genotype of the *Mx* gene was recommended to enhance genetic resistance to avian influenza in Riau Province.

ACKNOWLEDGMENT

The authors gratefully acknowledge the Ministry of Religious Affairs of the Republic of Indonesia for financial support through the Litapdimas research grant.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest regarding the publication of this paper.

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