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Genetic Polymorphisms of Chicken Antiviral *Mx1* and *TVB* Genes in Indigenous and Giriraja Chicken

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ABSTRACT

Background: Recent developments in molecular genetics lead to addressing certain poultry diseases *via* breeding for disease resistance. The present study was carried to identify and compare the genetic polymorphism in Chicken *Mx1* and *TVB* genes among the indigenous and Giriraja chicken using PCR-RFLP technique.

Methods: Blood samples were collected from 50 indigenous and 50 Giriraja birds and DNA isolation was done by Phenol: Chloroform: Isoamyl alcohol method. PCR amplification of Chicken *Mx1* (exon 14) and Chicken *TVB* (exon 3) genes was carried out followed by RFLP analysis.

Result: PCR product sizes of 301 bp and 303 bp of *Mx1* and *TVB* genes, respectively were successfully amplified. RFLP analysis of *Mx1* gene with *Hyp*8I restriction enzyme revealed three genotypes AA, AB and BB. In indigenous birds genotypic frequencies of AA, AB and BB were 0.314, 0.493 and 0.194, respectively and gene frequencies were 0.56 and 0.44 for alleles A and B, respectively. In Giriraja birds, genotypic frequencies for AA, AB and BB were 0.27, 0.499 and 0.23, respectively and gene frequencies were 0.52 and 0.48 for alleles A and B, respectively. RFLP analysis of *TVB* gene with *Nla*III restriction enzyme revealed two genotypes *viz.,* AA and AB. In indigenous birds genotypic frequencies of AA and AB were 0.81 and 0.18, respectively and gene frequencies were 0.9 and 0.1 for alleles A and B, respectively. In Giriraja birds genotypic frequencies for AA and AB were 0.774 and 0.211, respectively and gene frequencies were 0.88 and 0.12 for alleles A and B, respectively.

Key words: Disease resistance, Genetic polymorphisms, *Mx1*, *TVB*, Viral diseases.

INTRODUCTION

Continuous selection for rapid growth and/or more egg production has resulted in the loss of disease resistance and overall immune-competence (Knap and Bishop, 2000). Currently, various contagious disease outbreaks cause severe production problems resulting in enormous economic losses in poultry industry. The commercial birds are losing their genetic resistance to various diseases due to continuous selection for production traits. The contemporary chicken is probably developed from its main wild ancestor, the red jungle fowl (*Gallus gallus*) and distributed worldwide which lead to adaptation of the birds to different environments. Since the process of adaptation of indigenous chicken to often harsh and extreme environmental conditions demanded positive selection towards enhanced immune resistance, these birds are now most likely carrying fixed alleles that determine their immune-competence (Ewald *et al*., 2011). The *Mx* gene found in various organisms, including human, vertebrates, fish and yeast (Ko *et al*., 2002; Wantanabe, 2007) have been reported to promote antiviral activity by inhibiting replication of various viruses. Ko *et al*. (2002) also reported that the chicken *Mx* gene was highly polymorphic and that the Mx alleles of some breeds of chicken had activity against influenza virus and vesicular stomatitis virus (VSV). Chickens have a single Mx gene that is induced by type I interferon (Schumacher *et al*., 1994) and is located on chicken chromosome 1 having 14 exons spanning 20767 bp (Hassanane *et al.,* 2018). Avian Leukosis Virus (ALV) is a retrovirus and is classified into six major subgroups (A to E and J) based on virus and cell receptor interaction patterns. Among these, one subgroup (E) is endogenous encoded by genes within the chicken genome, whereas all other subgroups (A, B, C, D and J) are exogenous (Nair and Fadly, 2013). The subgroup specific surface receptors on host cells that mediate or block the entry of all ALV subgroups are determined by four autosomal tumor viral loci *viz*., TV-A, TV-B, TV-C and TV-J. The receptors of ALV subgroups A, C and J are encoded by TV-A, TV-c and TV-J loci, respectively. The TV-B locus is most complex and encodes receptors for B, D and E subgroups of ALV (Payne and Venugopal, 2000). The TVB locus consists of three different alleles; TVB*S1, TVB*S3 and TVB*R. TVB*S1 allele encodes receptors for B, D and E subgroups of ALV to support the viral entry and TVB*S3 allele encodes a receptor that permits viral entry for both B

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and D subgroups of ALV. Whereas, TVB*R allele encodes a defective incomplete receptor, due to a premature stop codon within its RNA sequence; the TVB*R encoded receptor permits no viral entry to any of the ALV subgroups (Barnard and Young, 2003). The present study was aimed to determine the genetic polymorphism of chicken antiviral *Mx1* and *TVB* genes in Indigenous and Giriraja chicken.

MATERIALS AND METHODS

The present work was carried out at Dept. of Animal Genetics and Breeding, Veterinary College, Hebbal, Bengaluru-560 024 during 2018-19. Venous blood was collected from 50 indigenous and 50 Giriraja birds. DNA was extracted from avian whole blood by using the conventional Phenol: Chloroform: Iso-amyl alcohol (P: C: I) method (Khosravinia *et al.*, 2007).

Amplification of exon 14 region of *Mx1* gene was carried out by employing published primers *viz*., Forward: 5′GCACT GTCACCTCTTAATAGA 3′ and Reverse: 5′ GTATTGGTAGG CTTT GTTGA 3′ (Sironi *et al*., 2010; Hassanane *et al*., 2018). Amplification of exon 3 region of *TVB* gene was carried out by employing published primers *viz*., Forward: 5′ ACCCC TTCTTGCAGGCACCTATGA 3′ and Reverse: 5′ GGATG CTGTGCTGCGTGGAGA 3′ (Zhang *et al*., 2005; Shavakand, 2011; Liao *et al*., 2014; Sulimova *et al*., 2017).

The amplification for both the genes were performed in a total volume of 25 µl consisted of 12.5 µl of Red PCR master mix, 1 µl (10 pmol/ µl) each of forward and reverse primer, 9.5 µl of PCR grade water and 1 µl of template DNA. The PCR reaction was carried out with an initial denaturation temperature of 95°C (5 min), 35 cycles of 95°C (60 sec), 49°C and 65.6°C (60 sec) for *Mx1* and *TVB* genes, respectively and 72°C (60 sec), followed by final extension at 72°C (10 min). The PCR amplified products were allowed to run on 1.5 per cent agarose in parallel with 100 bp DNA ladder and photographed under gel documentation system (Bio rad Molecular imager Gel Doc XR+, USA).

The PCR products of the *Mx1* and *TVB* genes were digested with *Hyp*8I and *Nla*III restriction enzymes, respectively. The digestion was done in a total volume of 30 µl which consisted of 2 µl of 10X buffer, 10 µl of PCR amplicon, 1 µl of RE and 17 µl of Nuclease free water (NFW) with incubation at 37°C for 3 hours and inactivation at 80°C for 20 min for *Mx1* gene and incubation at 37°C for 5 min and inactivation at 80°C for 5 min for *TVB* gene. The RE digested products were resolved on two per cent agarose gel agarose in parallel with 100 bp DNA ladder and photographed under gel documentation system (Bio rad Molecular imager Gel Doc XR+, USA).

Based on visualization of different band patterns, genotypes were determined. The allele number, allele frequency, genotype frequency, observed and expected heterozygosity and Chi square were calculated.

RESULTS AND DISCUSSION

In the present study, Phenol: Chloroform: Iso-amyl alcohol (25:24:1) method gave optimum results at an incubation temperature of 55°C for overnight incubation. This procedure was found efficient in terms of cost and time when compared to other procedures as given by Plotsky *et al*. (1995). About 50 -150 µg of DNA was obtained per 50 µl of venous blood during this study. However, 132 µg of DNA per 28 µl and 4.99±0.01 µg of DNA per µl of whole blood were obtained by Khosravinia *et al*. (2007) and Pirany (2005). The variation in genomic DNA yield could be attributable to the laboratory environment and handling of the materials.

The PCR amplification of *Mx1* and *TVB* genes resulted in a single amplified product of 301 and 303 base pairs, respectively, in all the samples of indigenous and Giriraja birds (Fig 1, 2a and 2b). Similar size of amplified product for *Mx1* gene was reported by Hassanane *et al*. (2018) in Egyptian chicken but in contrary to the present study a 299 bp of amplified product was reported by Sironi *et al*. (2010) in commercial broilers and Pagala *et al*. (2013) in Tolaki chicken. For *TVB* gene, Zhang *et al*. (2005), Shavakand (2011), Liao *et al*. (2014), Sulimova *et al*. (2017) and Kaya *et al*. (2020) obtained a PCR product of 303 bp which is similar to the present study.

The PCR-RFLP analysis of Chicken *Mx1* gene (exon 14) using Hyp8I restriction enzyme yielded three patterns in Indigenous and Giriraja birds which corresponded to three genotypes *viz*., AA, AB, BB (Fig 3). The frequency of AA, AB

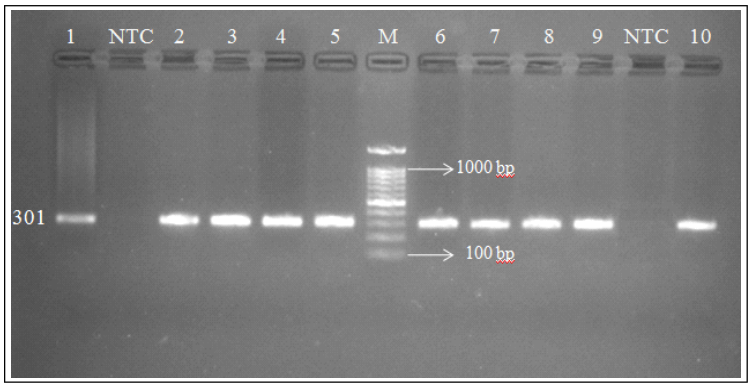

Fig 1: Agarose gel (1.5%) picture showing PCR amplicons of *MX1* (exon 14) gene in indigenous (Lane 1-5) and Giriraja chicken (Lane 6 - 10).

and BB genotypes were 0.314, 0.493 and 0.194, respectively in Indigenous birds and 0.270, 0.499 and 0.230, respectively in Giriraja birds. The gene frequencies were 0.56 and 0.44 in Indigenous birds and 0.52 and 0.48 in Giriraja birds for A and B alleles, respectively. In agreement with the present study, Hassanane *et al*. (2018) also reported three genotypes AA, AG and GG with genotypic frequencies of 0.44, 0.45 and 0.11, respectively and with gene frequencies of 0.67 and 0.33 for A and G alleles, respectively. Pagala *et al*. (2013) also reported three genotypes AA, AG and GG with genotypic frequencies of 0.617, 0.260 and 0.126, respectively and with gene frequencies of 0.74 and 0.26 for A and G alleles,

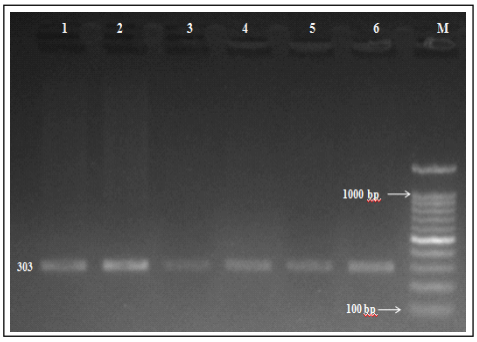

Fig 2a: Agarose gel (1.5%) picture showing PCR amplicons of *TVB* (exon 3) gene in indigenous chicken.

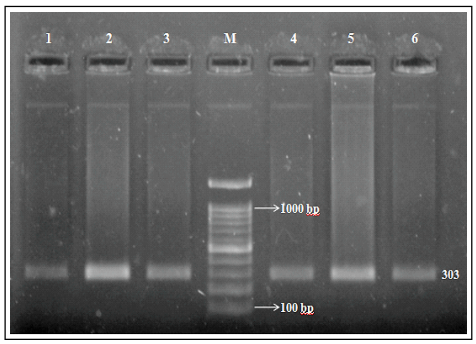

Fig 2b: Agarose gel (1.5%) picture showing PCR amplicons of *TVB* (exon 3) gene in Giriraja chicken.

respectively. However, in the present study the heterozygous genotypes were more in Indigenous and Giriraja birds which are contrary to the results of Pagala *et al*. (2013). PCR-RFLP analysis of intron 12-13 region of *Mx1* gene with *Hpy8*I restriction enzyme revealed three genotypes, AA, AG and GG with frequency of 21.33, 58.67 and 8.67 per cent in Indigenous chickens of Kenya (Okumu, 2016).

The PCR-RFLP analysis of Chicken *TVB* gene (exon 3) with NlaIII restriction enzyme yielded two patterns in Indigenous and Giriraja birds which corresponded to two genotypes *viz*., AA and AB (Fig 4). The frequency of AA and AB genotypes were 0.81 and 0.18, respectively in Indigenous birds and 0.774 and 0.211, respectively in Giriraja birds. The gene frequencies were 0.9 and 0.1 in Indigenous birds and 0.88 and 0.12 in Giriraja birds for alleles A and B, respectively. In contrary to the present study, Zhang *et al*. (2005), Shavakand (2011) and Kaya *et al*. (2020) reported three genotypes S1S1, S1S2 and S2S2. Liao *et al*. (2014) reported 4 genotypes *viz*., S1S1, RR, S1R and S1S3 in Chinese chicken. The S1S1 genotype was detected in all Chinese chickens surveyed with an average frequency of 0.87, RR genotype was detected in JNBRC, CB07, CB12, CB14 and CB15 with frequencies of 0.03, 0.03, 0.11, 0.09 and 0.15, respectively. The heterozygote S1R was detected in 6 of the 10 chines local chickens and 4 of the 15 commercial broilers tested with the average frequencies of 0.12 and 0.08, respectively. The heterozygote S1S3 was detected in 4 of the 10 Chinese local chicken breeds, including XHC, NDHC, JNBRC and HYBC with the frequencies at 0.05, 0.13, 0.14 and 0.03, respectively.

The observed and expected heterozygosities and chi square value for Indigenous and Giriraja birds is presented in Table 1. The observed and expected heterozygosities for Chicken *Mx1* gene were 0.52 and 0.493, respectively in Indigenous birds, and 0.56 and 0.499, respectively in Giriraja birds. Whereas, the observed and expected heterozygosities for Chicken *TVB* gene were 0.2 and 0.18, respectively in Indigenous birds and 0.24 and 0.211, respectively in Giriraja birds. In contrary to the present study, Shavakand (2011) reported lowest observed and expected heterozygosities of

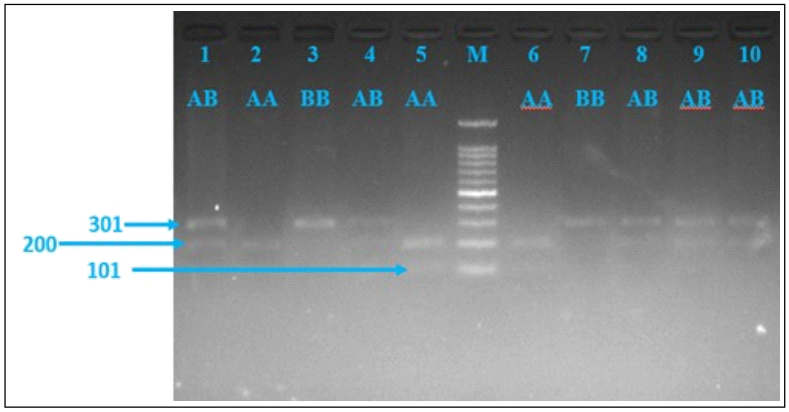

Fig 3: Agarose gel (2%) picture showing PCR-RFLP pattern of *MX1* (exon 14) gene in indigenous (Lanes 1-5) and Giriraja (Lanes 6-10).

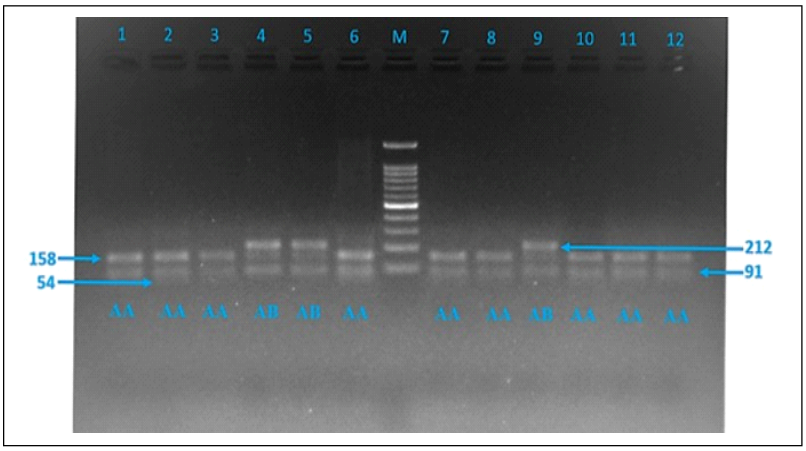

Fig 4: Agarose gel (2%) picture showing PCR - RFLP pattern of TVB (exon 3) gene in indigenous (Lanes 1-6) and Giriraja (Lanes 7-12) chicken.

Table 1: Gene and genotype frequencies, observed and expected heterozygosity and χ^2 value in indigenous and Giriraja chicken.

Gene	Breed	Allele frequency		Genotypic frequency			Observed hetero-zygosity (Ho)	Expected hetero-zygosity (He)	Chi-square value
		A	B	AA	AB	BB			
MX1	Indigenous	0.56	0.44	0.314	0.493	0.194	0.52	0.493	0.152^{NS}
	Giriraja	0.52	0.48	0.27	0.499	0.23	0.56	0.499	0.742^{NS}
TVB	Indigenous	0.9	0.1	0.81	0.18	0.01	0.2	0.18	0.617^{NS}
	Giriraja	0.88	0.12	0.774	0.211	0.014	0.24	0.211	0.930^{NS}

0.087 and 0.1, respectively in five chicken populations (Silkies, Taiwanese cross, Lohmann Brown, Lohmann White and Agassiz Cross).

In the present study, the Chi-square test indicated that the studied indigenous and Giriraja populations were in Hardy-Weinberg equilibrium. Shavakand (2011) reported that Silkies, Lohmann Brown and Agassiz cross populations were in Hardy-Weinberg equilibrium which is in agreement with the present study, whereas Taiwanese cross population was deviated from Hardy-Weinberg equilibrium which is in contrary to the present study.

The contrasting report in the present study compared to the earlier reports may be attributed to the genetic makeup, the breeding programme followed and the sample size of birds involved.

CONCLUSION

The present study successfully demonstrated the genetic variability in *Mx1* locus (exon 14) and *TVB* locus (exon 3) in both indigenous and Giriraja birds. These two genes may be considered as the potential gene marker for within breed selection for disease resistance against viral diseases in chicken provided that association is established between genotypes and disease resistance traits. Thus, there is a need to conduct an extensive study within breed for association analysis between these gene variants and disease resistance traits.

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Conflict of interest

All authors declare that they have no conflict of interest.

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