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Research Article

Polymorphism of Myxovirus Resistance (Mx) Gene in Four Nigerian Chicken Genotypes

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Abstract

The study investigated the polymorphism of myxovirus resistance (Mx) gene in four Nigerian chicken genotypes. A total of 200 chickens comprising of 50 Normal feathered, 50 Frizzle feathered, 50 Naked neck, and 50 Noiler chickens. The chickens were kept for 16 weeks during which feed and water were given ad libitum. Other management practices were carried out. Blood samples were collected from the chickens via the wing vein using 2ml syringe into an EDTA bottle for DNA extraction. Polymerase Chain Reaction (RCR) was carried out using the DNA extracted and primers of Mx gene. PCR amplicons were sequences and sequences subjected to analysis. Results obtained showed the highest genetic distance is between the Frizzled feather and Naked neck (0.1537) and the least is between the Noiler and the Normal feather (0.0755). Nucleotide diversity with JC (PiJC) obtained for Normal feathered, Naked neck, Noiler and Frizzled feather genotype were 0.01005, 0.05868, 0.01973 and 0.17961. Gst value obtained (0.04437) indicated that 95.56% genetic variation within populations and only 4.44% between populations. The estimated Nm value (5.38) suggested genetic differentiation among populations of Nigerian chicken genotypes impeded by high gene flow. This could be further supported by the result obtained in the genetic distance which suggested relatedness among the chicken populations studied.

Keywords: Myxovirus Resistance Gene; Polymorphisms; Noiler; Naked Neck; Normal Feather; Frizzled Feather

Introduction

Due to the rapid spread of infections among chickens, breeders have begun to focus on genes associated with the innate immune system, such as Myxovirus resistance protein gene with birds showing different susceptibilities [1]. These genes play an active role in mitigating the incidence of infections. Myxovirus resistance (Mx) gene has been shown to have an inhibitory effect on influenza virus. It has a direct antiviral activity that inhibits a wide range of viruses by blocking an early stage of the viral replication cycle [2]. Most of the available reports have demonstrated the role of the chicken Mx gene G2032A (S631N) in antiviral activities, particularly against influenza virus. Different studies have assessed the improvement made so far in the Nigerian local chickens. This study was carried out to evaluate the single nucleotide polymorphisms (SNPs) gene flow, genetic differentiation, the sequence, haplotype and nucleotide diversity in Mx gene of Nigerian local and exotic chickens. Therefore, the present study was aimed to investigate the polymorphism of Myxovirus resistance protein gene in Nigerian chickens.

Materials and Methods

The study was carried out at the Agricultural Research and Demonstration Farm and Animal Science Laboratory, Faculty of Agriculture, University of Port-Harcourt. A total of 200 chickens were used in this study which included fifty (50) Normal feathered chicken, fifty (50) Frizzle feathered chicken, fifty (50) Naked Neck, and fifty (50) Noiler chickens. The birds were kept for 12 weeks during which feed and water was provided ad libtum. Vaccinations and other medications were administered at the right time. At the end of the research, blood samples were collected from the chickens via the wing vein using 2ml syringe into an EDTA bottle for

DNA extraction at the Laboratory of Animal Science Department, Faculty of Agriculture, University of Port Harcourt, Nigeria.

DNA was extracted from the chickens using the Quick – DNA Miniprep kit from Zymo Research, USA according to the manufacturer's protocol. The quantity and quality of the DNA extracted were assessed using a nano spectrophotometer and gel electrophoresis. Polymerase chain reaction (PCR) was done to amplify a DNA fragment of about 284 bp region of the 5′ untranslated region and partial promoter of the chicken Mx gene. The selected primers (forward primer: 5′-ACCTGTGCCATCTGCCCTCTGA-3′) and (reverse primer: 5′-CACAGCAAGGAGAAACAATTAACTACAT-3′). The PCR cocktail and conditions have being previously reported by Agaviezor., *et al*. [3].

Statistical analysis

Sequences were edited and aligned using Mega 6.0 [4]. Polymorphism, genetic diversity and similarity indices, gene flow and genetic differentiation were determined using Mega 6.0 [4] and DNAsp Version 5 [5].

Results

Gel electrophoresis picture of PCR products of the Mx gene in Nigerian chickens is shown in figure 1. The Mx gene was observed in most chickens which showed accurate amplified Mx gene.

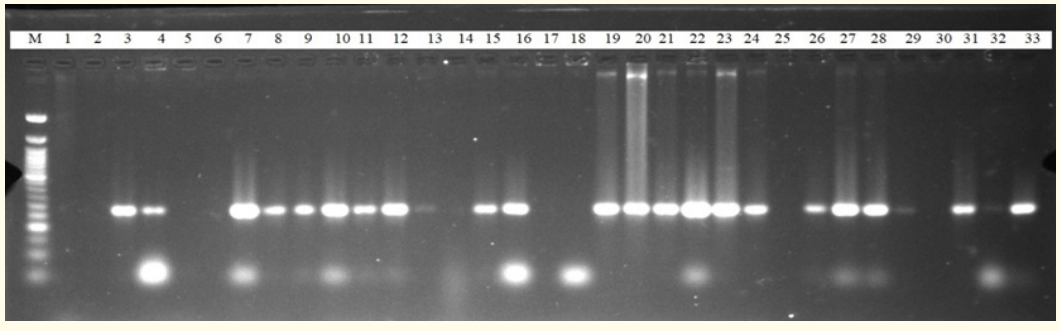

Figure 1: Gel electrophoresis showing PCR products of Mx gene in Nigerian chickens.

Table 1 shows the genetic distance of Myxovirus resistance protein gene among Nigerian chicken genotypes studied. Genetic distance of 0.2062 was observed in Frizzled feather chicken, 0.079 for Noiler chicken, 0.1246 for Naked neck chicken and 0.0763 for Normal feathered chicken.

Genotypes	Genetic distance
Frizzle feather	0.2062
Noiler	0.0779
Naked Neck	0.1246
Normal	0.0763

Table 1: Genetic distance in Myxovirus resistance protein gene in studied chickens.

Table 2 shows the genetic distance in myxovirus resistance protein gene between Nigerian chicken genotypes studied. Genetic distance between the Frizzled feather and Noiler is 0.1250, between the Frizzled feather and the Naked neck is 0.1537, between the Frizzled feather and the Normal feather is 0.1242, between the Noiler and the Naked neck is 0.1106, between the Noiler and the Normal feather is 0.0755, between the Naked neck and the Normal feather is 0.1026. The highest genetic distance is between the Frizzled feather and Naked neck (0.1537) and the least is between the Noiler and the Normal feather (0.0755).

	Frizzle feather	Noiler	Naked Neck	Normal
Frizzle feather	1			
Noiler	0.1250	1		
Naked Neck	0.1537	0.1106	1	
Normal	0.1242	0.0755	0.1026	1

Table 2: Genetic distance in Myxovirus resistance protein gene between the chicken genotypes studied.

Figure 2 shows the phylogenetic tree of myxovirus resistance protein gene of four Nigerian chicken genotype studied which translates the genetic relatedness in the Mx gene among the genotypes studied. The relatedness differs from each other as were revealed from the result.

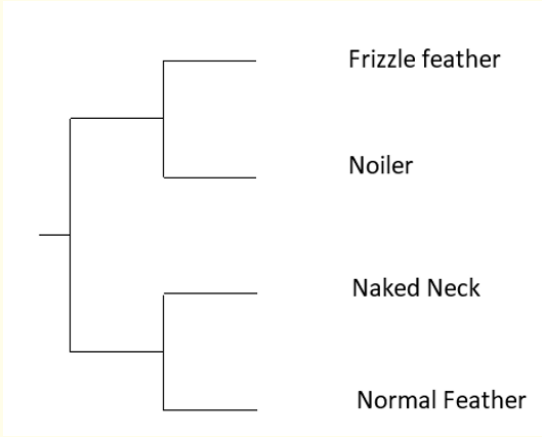

Figure 2: Dendogram showing genetic relatedness in the Mx gene among strains of chickens studied.

Table 3 shows the myxovirus resistance protein gene sequences, number of segregating sites (S), haplotype number (h), haplotype diversity (Hd), average number of differences (K), nucleotide diversity (Pi), and nucleotide diversity with JC (PiJC) in the chicken genotype studied. Number of sequences obtained for Normal feathered genotype was 7, Naked neck genotype was 6, Noiler was 5 and Frizzled feather was 2, while the total number obtained was 20. Selected region was 1-256. Number of sites was 256. Total number of sites excluding gaps was 144. Sites with alignment gabs were not considered. Number of segregating sites (S) was 4 for Normal feathered genotype, 19 for Naked neck genotype, 6 for Noiler, and 23 for Frizzled feather while number of variable sites is 36. In addition, the number of haplotypes (h) were also found to be 3 in Normal feathered genotype, 6 was obtained for Naked neck, 5 was obtained for Noiler while 2 was obtained for Frizzled feather. Haplotype diversity (Hd) obtained in these four chicken genotypes were found to be 0.714, 1.000, 1.000, 1.000 for Normal feather genotype, Naked neck, Noiler and Frizzled feathered genotype respectively.

The average number of differences (K) was found to be 1.428, 8.000, 2.800, 23.000 for Normal feather, Naked neck, Noiler and Frizzled feather genotypes respectively. Nucleotide diversity (Pi), were also found to be 0.009 for Normal feathered genotype, 0.055 for Naked neck, 0.019 for Noiler and 0.159 for Frizzled feathered genotype. Nucleotide diversity with JC (PiJC) obtained for Normal feathered, Naked neck, Noiler and Frizzled feather genotype were 0.010, 0.058, 0.019 and 0.179. Meanwhile, the total data estimate were 20, 36, 13, 0.931, 5.863 and 0.040 for the number of sequences, number of segregating sites (S), number of haplotypes (h), haplotype diversity (Hd), average number of differences (K) nucleotide diversity (Pi).

		Population			
	Normal	**Naked Neck**	**Noiler**	**Frizzle Feather**	**Total data estimate**
Number of sequences:	7.000	6.000	5.000	2.000	20.000
Number of segregating sites, S:	4.000	19.000	6.000	23.000	36.000
Number of haplotypes, h:	3.000	6.000	5.000	2.000	13.000
Haplotype diversity, Hd:	0.714	1.000	1.000	1.000	0.931
Average number of differences, K:	1.428	8.000	2.800	23.000	5.863
Nucleotide diversity, Pi:	0.009	0.055	0.019	0.159	0.040
Nucleotide diversity with JC, PiJC:	0.010	0.058	0.019	0.179	0.040

Table 3: Sequence, haplotype and nucleotide diversity of myxovirus resistance protein gene of Nigerian chicken genotypes.

Table 4 shows the Genetic differentiation estimates in myxovirus resistance protein gene of four Nigerian chicken genotypes. The value for the genetic differentiation for Hs, Hst, Ks, Ks*and Z were 0.880, 0.054, 5.900, 1.295 and 82.294 respectively while the value for Snn, Kst, Kst* and Z*: where 0.249, -0.006, 0.155 and 4.064 respectively. The genetic differentiation estimates in Mx gene of Nigerian local and exotic chickens for Z: was highest followed by Ks, Z* Ks*, Hs, Snn, Kst* and Hst while the least is Kst which has a negative value as shown in table 2 below.

Hs, Hst - measure genetic differentiation based on haplotype statistics, Ks, Kst, Snn, Z-measure genetic differentiation based on nucleotide statistics. Chi2: 8.000 P-value of Chi2: 0.1562 ns; (df = 5), ns, not significant; *, 0.01 < P < 0.05; **, 0.001 < P < 0.01; ***, P < 0.001

Table 5 shows the gene flow estimates in myxovirus resistance protein gene of Nigerian chicken genotypes. The haplotype data

Parameters	Values
Hs:	0.88095
Hst:	0.05434
Ks:	5.90000
Ks*:	1.29501
Z:	82.29464
Snn:	0.24940
Kst:	-0.00628
Kst*:	0.15574
Z*:	4.06436

Table 4: Genetic differentiation estimates in myxovirus resistance protein gene of Nigerian chicken genotypes.

Haplotype Data Information (6)		Sequence Data Information (8)			Lynch and Crease (7) (with Jukes and Cantor correction)		Hudson, Slatkin and Maddison (9)	
Gst:	Nm:	DeltaSt:	GammaSt:	Nm:	Nst:	Nm:	Fst:	Nm:
0.044	5.380	0.009	0.256	0.720	-0.035	-7.230	-0.031	-8.110

Table 5: Gene Flow Estimates in Myxovirus resistance protein gene of Nigerian chicken genotypes.

Discussion

The results obtained in this study showed some variations across the parameters studied. Variations in polymorphic sites were observed across the poultry breeds. These variations could be as a result of mutations and adaption over the years. Genetic difference in myxovirus resistance protein gene among strains of chicken studied ranges from 0.073-0.15. This result is supported by [10] who observed values ranging from 0.074-0.519. Variations were seen in areas such as number of sites which ranges from 0-256, this result is not in accordance with that reported by Agaviezor and Chukwuemeka [3] who recorded number of segregating site which ranged from 41-174. The genetic distance of the chicken genotypes studied showed significant variation. Frizzled feather had the highest value which was 0.2062, Noiler had value of 0.0779, Naked neck had the value of 0.1246 and Normal feather had the value of 0.0765 which was the lowest recorded value.

The phylogenetic tree showing genetic relatedness in the myxovirus resistance protein gene among chickens studied revealed low genetic distance indicating that the four strains are closely related. This result is similar to that reported by Nweke-Okorocha., *et al*. [10]. The number of sequences in this study ranges from [2-7] which is indicative of a positive selection across the organisms and is supported by Ilori., *et al*. [11] and Nweke-Okorocha., *et al*. (10). Uberu., *et al.* [12] also recorded a higher number of sequencing information [6] on Gst and Nm were 0.044 and 5.380 respectively while the sequence data information on DeltaSt, GammaSt and Nm: were 0.009, 0.256 and 0.720 respectively as shown in Table 5. The Lych and Crease [7] (with Jukes and Cantor correction) for Nst and Nm: were -0.035 and -7.230 respectively while the Hudson., *et al*. (1992) values for Fst: and Nm: were -0.031 and -8.110 respectively as shown in Table 5. The haplotype data information provided by Nei (1973) among population of ducks for Nm: was higher than those reported by Nei [8], Lych and Crease [7] and Hudson., *et al*. [9] while the value for GammaSt: was highest followed by Gst, DeltaSt, Fst while the least was Nst as shown in Table 5 below.

compared to what was obtained in this study. The number of segregating sites in this study ranges from [4-23] which is supported by Ilori., *et al.* [11] and Nweke-Okorocha., *et al*. [10] showed that the segregating sites obtained in this study is satisfactory. However, Gao., *et al.* [13] Okafor., *et al.* [14] and Uberu., *et al.* [12] reported higher number of segregating sites. The number of segregating sites is used to estimate mutation rate assuming no selection.

The number of haplotype obtained in this study ranges from [2-6] which is supported by what Nweke-Okorocha., *et al*. [10], Ilori., *et al*. [11], Okafor., *et al.* [14], Gao., *et al.* [13], and Agaviezor., *et al.* [15] reported from their respective findings. The haplotype diversity in this study ranged from 0.71429-1.00000 for the four Nigerian chicken genotypes used in this study which lies within the appropriate range of haplotype diversity. The average number of differences, K for this study ranges from 1.4-23 and it's supported by Ilori., *et al.* [11]. Nweke-Okorocha., *et al*. [10] and Okafor., *et al.* [14] reported higher values which could be linked to the gene of study. The value of the Nucleotide diversity, Pi of this study ranges from 0.00992-0.15972. This is supported by Ilori., *et al*. [11], Agaviezor., *et al*. [15], Uberu., *et al.* [12] and Nweke-Okorocha., *et al*. [10]. Genetic differentiation estimates in Myxovirus resistance protein gene of Nigerian chicken genotypes. Genetic differentiation occurs when there is restricted gene flow between populations. The value for the genetic differentiation for Hs, Hst, Ks, Ks*and Z were

0.88095, 0.05434, 5.90000, 1.29501 and 82.29464 respectively while the value for Snn, Kst, Kst* and Z*: where 0.24940, -0.00628, 0.15574 and 4.06436 respectively. The genetic differentiation estimates in myxovirus resistance protein gene of Nigerian chicken genotypes for Z: was highest followed by Ks, Z* Ks*, Hs, Snn, Kst* and Hst while the least is Kst which has a negative value. The Hs of this study was higher than the result obtained by Wang., *et al*. [16].

The percentage of polymorphism, haplotype diversity and nucleotide diversity are used to evaluate population genetic diversity [17]. High levels of genetic diversity are indicative of the strong viability and adaptability of population [18]. High genetic diversity between populations may increase fitness in populations to changing conditions [19]. Genetic diversity increases the ability of species to colonize on a short-term ecological timescale by increasing the possibility of population survival, growth and reproduction under novel environments [20]. Gene flow which is movement of genes may in some cases allow small fragments of DNA pass from one individual directly into the germline of another, perhaps transduced by a pathogenic virus or other vector, or deliberately via a human transgenic manipulation [21]. A high rate of gene flow within individual species will increase the rate of lineage sorting between different species [22] and thereby reduce the level of introgression [23].

Gst which is an estimate that measures genetic differentiation is inversely proportional to gene flow. The low Gst values therefore recorded is explained by the level of polymorphism [15]. Gst value obtained in this study was 0.04437 which indicates that 95.56% genetic variation is within populations and only 4.44% between populations. This result is lower than what Agaviezor., *et al*. [15] and Wang., *et al*. [16] reported respectively and that could be as a result of environmental factors. The estimated Nm value was 5.38 which suggested that genetic differentiation among populations of Nigerian chicken genotypes is impeded by high gene flow. This could be supported by the result obtained in the genetic distance among the chicken populations studied.

The values obtained from the Sampling variance of k (no recombination), Vs (k), Sampling variance of k (free recombination), Vs (k), Variance of theta (no recombination) and Variance of theta (free recombination) of this study lies within the value obtained by Amr and Satish, [24]. However, Marzouk [25] obtained higher value from Stochastic variance of k (no recombination), Vst (k), Sampling variance of k (no recombination), Vs (k), Total variance of k (no recombination), V (k), Stochastic variance of k (free recombination), Vst (k), Sampling variance of k (free recombination), Vs (k), Total variance of k (free recombination), Theta (per sequence) from S, Theta-W, Variance of theta (no recombination) and Variance of theta (free recombination), which could be as a result of the gene under study or the experimental animal.

Conclusion

This study has uncovered the polymorphism of myxovirus resistance (Mx) gene in four Nigerian chicken genotypes. The variation in the various indices shows the level of improvement done so far in the Nigerian indigenous local chicken breeds. The information obtained from this study would be very useful in further planning, conservation and genetic improvement of Nigerian local chicken through selection and very essential in understanding the myxovirus resistance protein gene and its resistance to infections and viral diseases especially the Newcastle disease.

Conflict of Interest

The authors declare that no financial interest or any conflict of interest exists.

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