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Avian Influenza Resistant *Gene* (*Mx*) and Its Diversity in Chicken and Duck

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Abstract

Poultry sector has been facing threat of highly pathogenic avian influenza (HPAI) since 2007 in Bangladesh. The chicken Mx protein has been reported to exhibit antiviral activity against influenza virus. Therefore, targeting *Mx* gene may be an approach for development of AI resistant poultry. However, there is scarcity of information about the *Mx* gene and its diversity in chicken and duck in Bangladesh. To enumerate *Mx* gene and its diversity a total of 60 blood samples were collected from six chicken groups (Rhode Island Red, White Leghorn, White Rock, Barred Plymouth Rock, Necked Neck & Hilly) and four duck groups (Pekin, Rupali, Nageshwari & Common Deshi) from Bangladesh Livestock Research Institute, Nageshwary, Kurigram district and Natore Sadar, Natore district. Two sets of primers were used. Of them one set for *Mx* gene detection by PCR and another set for *Mx* gene detection and its diversity analysis by polymerase chain reaction restriction fragment length polymorphism (PCR-RFLP) using *Rsa*I and *Ssp*I restriction enzymes. The *Mx* gene detection with one set primer was found to vary from 60.0-100% (average 77.8%) in chicken and 0-40% (average 43.6) in duck. However, with another set of primer detection rate was 40-100 and 66.7-100% in chicken and duck, respectively. PCR-RFLP analysis of 27 samples revealed that tested samples contain homozygous resistant (R/R), homozygous sensitive (S/S) and heterozygous (R/S) *Mx* allelic gene. In chicken the R/R, S/S and R/S *Mx* allelic gene was 38.9, 33.3 and 27.0% respectively; while in duck it was 11.1, 66.7 and 22.2%, respectively. Ducks were found more sensitive then tested chicken. *Mx* gene diversity was found to exist not only among the groups but also within the group of chicken and duck. To the best of our knowledge this is the first report on *Mx* gene study in Bangladesh covering both chicken and duck.

Keywords

Mx Gene, Diversity, Chicken, Duck, Avian Influenza

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1. Introduction

Type I interferon (IFN) produced by virally-infected cells play a crucial role in the host's early defence against viruses by helping in expression of a number of host genes, including *Myxovirus* resistant gene (*Mx*) (Muller *et al.,* 1994; Horisberger, 1995). The specific function of the Mx protein in defence against viruses is the result of a direct interaction between the carboxyl terminus of the Mx protein of specific species of pathogenic viruses (Lee and Vidal 2002). Mx protein has been found in many organisms, including yeast, mouse, fish, birds, and mammals (Aebi *et al.,* 1989; Meier *et al.,* 1990; Rothman *et al.,* 1990; Bazzigher *et al.,* 1993). Most species have two *Mx* genes which code for proteins that

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accumulate in either the nucleus or the cytoplasm of cell. Mouse and rat Mx1 proteins are located in the nucleus, whereas most other Mx proteins are found in the cytoplasm (Haller *et al.,* 2007). The nuclear mouse Mx1 protein primarily inhibits the replication of *Orthomyxoviruses,* including influenza viruses (Garber *et al.,* 1991; Haller *et al.,* 1995) and the cytoplasmic Mx2 protein mainly inhibits the vesicular stomatitis virus (Zurcher*et al.,* 1992; Jin *et al.,* 1999). Cytoplasmic Mx proteins such as the human MxA or bovine Mx1 not only confer antiviral activity against influenza viruses but also inhibit many unrelated viruses (Pavlovic *et al.,* 1992; Schneider *et al.,* 1994; Schwemmle *et al.,* 1995; Landis *et al.,* 1998; Baise *et al.,* 2004). The *Mx* gene was first identified in fowl in 1980 when research on *Mx* genes revealed host defence mechanisms against influenza virus infections (Livant *et al.,* 2007). In duck and chicken, only one Mxprotein was identified. The chicken Mx protein exhibit antiviral activity against the influenza virus and the recombinant vesicular stomatitis virus (VSV) (Ko *et al.,* 2002). It is also reported that naturally, native chicken has ability to resist the virus controlled by antiviral *Mx* gene (Sartika *et al.,* 2011). However, Benfield *et al.,* (2008) reported that breed Shamo, which has an Asparagine at amino acid 631 is void of activity against the H5N1. Each chicken's ability to resist the virus differs from one chicken to another and the antiviral gene control it. In chickens, the *Mx* gene is located in chromosome 1 in a 20767 bp fragment. It consists of 13 exons, with as many as 2115bp coding regions and 705 amino acids. Resistance against the AI virus was found at exon 13, nucleotide number 1892 where it undergoes alkaline transition mutation (single mutation) (Li *et al.,* 2007). Mutations in the *Mx* gene cause triple codon changes to 631, which mutates the amino acid asparagine (AAC/AAU) to serine (AGC/AGU). Ko *et al.,* (2002) showed that in many natural variations of chicken *Mx* gene, only the 631 (Ser to Asn) mutations was caused by a single nucleotide substitution in 2,032 point, which has antiviral activity. Chicken *Mx* gene is highly polymorphic, and that a single-nucleotide polymorphism affecting amino acid 631 determines antiviral activity (Janzen *et al.,* 2000). Therefore a mutation of the amino acid asparagine to serine, the resulting polymorphism in the *Mx* gene will cause to be susceptible to AI virus subtype H5N1 and AI virus attacks (Li *et al.,* 2007).

Livestock is an integral part of agricultural economy of Bangladesh. It provides food, nutrition, employment opportunity, income, draft power, fuel, manure, transport etc. Bangladesh Economic Review (2009) reported highest growth rate in livestock subsector than crop and fisheries. However, a declining growth rate was reported during 2006-2007 and 2007-2008 due to serious outbreak of highly pathogenic avian influenza (HPAI) in poultry. Native chicken (~140 million) and ducks (~38 million) are two important species of poultry distributed all over the country, source of protein among low income families, (Bhuiyan *et al.,* 2005& 2013), predominantly reared by women thus enhancing women empowerment. With the emergence of HPAI H5N1 in Bangladesh in 2007, conventional control strategies based on surveillance, stamping out, movement restriction and enforcement of biosecurity measures did not prevent the virus spreading and outbreak. Moreover, a new clade 2.3.2.1 emerged in January 2011. Later government of Bangladesh decided to allow vaccination on experimental basis in 2012 and still going on. To aid in HPAI control program, alternative and complementary ways of controlling of HPAI can be explored. The use of antiviral chemotherapy and natural compounds, avian-cytokines, RNA interference, genetic breeding and/or development of transgenic poultry may be the options (Abdelwhab and Hafez 2012). *Mx* gene may be the target one for genetic breeding and/or development of AIV resistant bird. Thus determination of *Mx* gene and its diversity in chicken and duck available in the country is significant. However, there is scarcity of published report about study on *Mx* gene in Bangladesh. In an initial study on *Mx* gene and its diversity in chicken we showed three types *Mx* gene allele biz. homozygous resistant (R/R), heterozygous (R/S) and homozygous sensitive (S/S) is existed in the sampled chicken population (Jahangir *et al.,* 2015).

2. Materials and Methods

2.1. Blood Sample Collection

A total of 60 blood samples were collected from ten groups (5 from each group) of chicken (*Gallus gallus*) and duck (*Anas platyrhynchos*) (Table 1). Of these 50 samples were obtained from Bangladesh Livestock Research Institute (BLRI), Savar, Dhaka, and 10 samples from Nageswary, Kurigram (n=5) and Natore Sadar, Natore (n=5). Chicken groups include Rhode Island Red (RIR), White Leg Horn (WLH), Barred Plymouth Rock (BPR), White Rock (WR), Necked Neck (NN) and Hilly (Hy) and the duck types include Pekin (Pk) Rupali (Rp), Nageshwary (Ny) Commom deshi (CD). About 4% triglyceride was used as an anticoagulant to collect blood. After collection blood was kept in ice box having ice pack and transferred to the laboratory of Animal Biotechnology Division, National Institute of Biotechnology (NIB) and stored at -20^{o}C until use. Laboratory analysis was done at NIB.

2.2. Preparation of Genomic DNA

The DNA was extracted from blood samples using the Phenol: chloroform: iso-amylalcohol method. The extraction

procedure was as follows: 200µl of blood was taken in a 1.5 ml micro centrifuge tube and 20µl of proteinase K was added and mixed well. To this, equal volume of Phenol: Chloroform: Iso-amyl alcohol (25:24:1) was added, then gently mixed and centrifuged at 8000Xg for 2 minutes under refrigerated condition. The upper aqueous phase was transferred to a new micro centrifuge tube and above procedure was repeated. The upper aqueous phase was transferred to a new micro centrifuge tube and equal volume of chloroform was added, then gently mixed and centrifuged at 8000Xg for 2 minutes under refrigerated condition. Three molar sodium acetate was added to one tenth of the volume of protein free DNA solution (upper aqueous phase) and two volumes of absolute ethanol was added and kept at –80°C for one hour followed by centrifugation at 15000Xg for 15 minutes. The DNA pellet was re-suspend in 70% ethanol and centrifuged at 15000Xg for 15 minutes and the DNA pellet was dried till no more ethanol left in the tube. DNA was suspended in 50µl of nuclease free water and stored at -20°C.

2.3. Primers

Two sets of primers were used in this study. One set designed by Sironi *et. al.*, (2010) was used to detect the *Mx* gene. These primers could amplify ~299bp fragments of *Mx* gene. Another set of mismatched primers designed by Seyama *et al.,* (2006) were used to amplify 100bp fragments of *Mx* gene followed by restriction enzyme analysis.

2.4. Polymerase Chain Reaction (PCR)

The PCR reaction mixture consisted of Genei red dye Master mix with enzyme12.5µl, forward and reverse primer each 1µl (20 pmole); template DNA 2µl and water 8.5µl. The cycling profile comprised an initial denaturing step for 5 min at 94°C, followed by 35 cycles of 94°C for 1 min, 60°C for 1 min, and 72°C for 1 min, and a final extension at 72°C for 5 min (Seyama *et al.,* 2006). For other primer specified conditions were used (Sironi *et al.,* 2010). Amplicons were analyzed by gel electrophoresis in a 2% agarose gel using TAE buffer and stained with ethidium bromide.

2.5. Restriction Fragment Length Polymorphism (RFLP)

The PCR products obtained using NE-F2 and NE-R2/R primers were digested with the restriction endonuclease *Rsa*I (Biolabs, USA) and those obtained using NE-F2 and NE-R2/S primers were digested with *Ssp*I (BioLabs, USA). The digestion reaction (10µl) consisted of nuclease free water 3µl, compatible 10X buffer 1µl, specific restriction enzyme 1µl and PCR product 5µl. The reaction mixture was incubated at 37°C water bath for 1hour. Upon digestion the products were electrophoresed in 3% agarose gel containing ethidium bromide. DNA was visualized with transilluminator.

2.6. Sequencing and Phylogenetic Analysis

PRC product was purified using EZ-10 Spin Column PCR Products Purification Kits-BS363 (Bio Basic Inc, Canada) according to manufacturer's instruction and stored at -20°C. Purified PCR product was sequenced by dideoxy chain terminating method using Dye Terminator Cycle Sequencing FS Ready Reaction Kit (Applied Biosystems, USA). The nucleotide sequencing reaction mixture contained 0.5µl purified PCR product, 2µl of primer (4 pmol/µl), 4µl of BigDye Terminator v3.1 (Cycle Sequencing Kit; ABI, Foster City, CA), and 4µl of BigDye® Terminator v1.1, v3.1 5X sequencing buffer (Applied Biosystems), and double distilled water amount necessary to make final volume of 20µl, according to the manufacturer's instructions. The sequence reaction was performed as follows: denaturation for 1 min at 94°C, followed by 25 cycles of PCR amplification, with each cycle consisting of 45 sec of denaturation at 96°C, 30 sec of annealing at 50°C and 4 min of elongation at 60°C. Then the product was purified by BigDye® XTerminator purification kit (Applied Biosystems, USA). The primer used forPCR was used in sequencing reaction. Sequencing was done from both forward and reverse direction. Nucleotide sequences were determined using an automated DNA sequencer 3110 Genetic Analyzer (Applied Biosystems, USA) and edited with SeqEscape V2.6 software. Multiple sequence alignments and processing were performed with the Molecular Evolutionary Genetics Analysis (MEGA) version 4.1.0 software (Tamura *et al.,* 2007) with an engine based on the ClustalW algorithm. Blast searches were used to retrieve the homologous sequences from the GenBank database. The phylogenetic analyses were performed using neighbor joining tree inference analysis.

3. Results

3.1. Amplification of ~299bp *Mx* Gene Fragment

A total of 55 DNA samples of chicken and duck were subjected to PCR with primer reported by Sironi *et al.,* (2010) and about ~299bp fragment (Fig. 1) of *Mx* gene was found in only 24 (43.6%) samples (Table 1). The overall detection of *Mx* gene by this primer set was 43.64%. Detection rate was found to ranges from 60.0-100.0% in chicken and 0-40% in duck. However, average detection rate was 77.8 and 10.7% in chicken and duck, respectively.

Table 1. Detection of *Mx* gene in chicken and duck.

Species & Source	Group of chicken and duck	No. of sample collected	No. of sample used in PCR	No. of sample positive	% positive
Chicken					
BLRI	White Leg Horn	5	5	4	80
	White Rock	5	5	4	80
	Rhode Island Red	5	5	4	80
	Barred Plymouth Rock	5	3	2	66.6
	Hilly	5	5	3	60
	Necked neck	5	4	4	100
Total		30	27	21	77.8
Duck					
BLRI	Pekin	5	5	0	0
	Rupali	5	5	0	0
	Nageshwari	5	5	2	40
	Common deshi	5	3	0	0
Others	Nageshwari (Kurigram)	5	5	1	20
	Common deshi (Natore)	5	5	0	0
Total		30	28	3	10.7
Overall		60	55		43.6

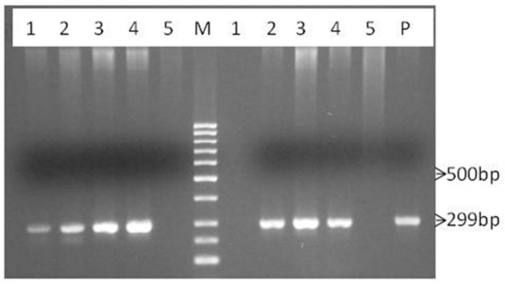

Fig. 1. Amplification of 299bp fragments of *Mx* gene from chicken and duck by Sironi's primer, Lane 1-5: Amplification of *Mx* gene from chicken (left) and duck (right), Lane M: Marker; Lane P: Positive control

3.2. Amplificationof 100bp Fragment of *Mx* Gene by Mismatched Primers

A total of 37 samples (27 from chicken and 10 from duck) were tested by PCR with mismatched primers NE-F2, NE-R2/R and NE-R2/Sreported by Seyama *et al.,* (2006). Overall 75.7% (28/37) samples were found positive (Table 2). The PCR products obtained using the mismatched primers were 100bp fragment of *Mx* gene (Fig. 2). Detection rate in chicken with mismatched primers was 40.0-100.0%. On the other hand in duck, the detection rate with NE-F2, NE-R2/R primerwas 100% while with NE-F2, NE-R2/S it was 66.6-100.0%.

Table 2. Amplification of 100bp *Mx* gene fragment from chicken and duck using mismatched primers.

Species & source	Group of chicken and duck	No. of sample tested by PCR	PCR result with NE-F2, NE-R2/R primers		PCR result with NE-F2, NE-R2/S primers	
			Positive	% positive	Positive	% positive
Chicken						
BLRI	White Leg Horn	5	3	60.0	4	80.0
	White Rock	5	4	80.0	4	80.0
	Rhode Island Red	5	3	60.0	3	60.0
	Barred Plymouth Rock	2	2	100.0	2	100.0
	Hilly	5	4	80.0	4	80.0
	Necked neck	5	2	40.0	2	40.0
Total		27	18	66.7	19	70.4
Duck						
BLRI	Pekin	2	2	100.0	2	100.0
	Rupali	3	3	100.0	3	100.0
	Nageshwary	2	2	100.0	2	100.0
	Common Deshi	3	3	100.0	2	66.7
Total		10	10	100.0	9	90.0
Overall		37	28	75.7	28	75.7

3.3. Diversity of *Mx* Gene in Chicken and Duck

A total of 27 PCR positive samples (18 from chicken and 9 from duck) with mismatched primers were used to determine the genetic variation/diversity of *Mx* gene in chicken and duck. PCR products obtained with NE-F2 & NE-R2/R primer were digested with restriction enzyme *Rsa*I while PCR products obtained with NE-F2 and NE-R2/S were digested with *Ssp*I. Both the enzymes would cut the 100bp PCR product into two 73 and 27bp length fragments. Three types of results viz. complete, partial or no digestion were observed with each of the restriction enzyme. Complete, partial and no digestion with *Rsa*I indicate sensitive (homozygous denoted

as S/S) variable (heterozygous denoted as R/S) and resistant (homozygous denoted as R/R), to influenza, respectively. While complete, partial and no digestion with *Ssp*I indicate resistant (homozygous denoted as R/R), variable (heterozygous denoted as R/S) and sensitive (homozygous denoted as S/S) to influenza, respectively. Interpretation of restriction enzyme analysis of 27 samples with both enzymes is presented in Table 3. Of 18 samples of chicken 38.9% (7/18) were found homozygous resistant, 27.0% (5/18) heterozygous and 33.3% (6/18) homozygous sensitive. On the other hand 11.1, 22.2 and 66.6% samples in duck were found homozygous resistant, heterozygous and homozygous sensitive, respectively. Comparatively ducks were found more sensitive.

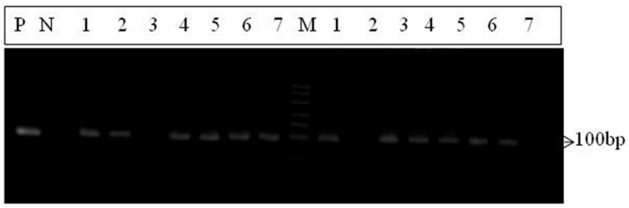

Fig. 2. Amplification of 100bp fragments of *Mx* gene by PCR from chicken. Lane 1-7 (left): Amplification by primer NE-F2 & NE-R2/R, Lane 1-7 (right): Amplification by primer NE-F2 and NE-R2/S, Lane M: Marker, Lane P: Positive control; Lane N: Negative control

Table 3. Diversity of *Mx* gene in chicken and duck

Species	Group of chicken and duck	No. of sample tested	Diversity of *Mx* gene		
			R/R	R/S	S/S
Chicken					
BLRI	White Leg Horn	3	1	1	1
	White Rock	4	1	1	2
	Rhode Island Red	3	0	1	2
	Barred Plymouth Rock	2	1	0	1
	Necked Neck	4	3	1	0
	Hilly	2	1	1	0
Total		18	7 (38.9%)	5 (27.0%)	6 (33.3%)
Duck					
BLRI	Pekin	2	0	0	2
	Rupali	3	0	1	2
	Nageshwary	2	0	0	2
	Common Deshi	2	1	1	0
Total		9	1 (11.1%)	2 (22.2%)	6 (66.7%)
Overall		27	8 (29.6%)	7 (25.9%)	12 (44.4%)

R/R: Homozygous resistant, R/S: Heterozygous, S/S: Homozygous sensitive.

3.4. Phylogenetic Analysis

One of the PCR products of native chicken necked neck was sequenced. Obtained 98bp sequence has 95% homology with the sequences of *Gallus gallus* located on the right side of the middle line drawn for necked neck (Fig.3). These sequences were derived from *Gallus gallus* clone EL494Mx1 (Accession No. 308212361), *Gallus gallus* Mx protein (Mx) gene (Accesssion No. 257219904), Gallus gallus isolate While Leghorn Mx protein (Mx) mRNA (Accession No. 146744131), *Gallus gallus* isolate WL_42Mx protein (Mx) mRNA (Accession No. 146744165), *Gallus gallus* isolate 25 breed White Leghorn MX (MX) gene (Accession No. 111182889) and *Gallus gallus*Mx protein mRNA (Accession No. 164551495). Sequences located left side of the middle line has 93% homology. They also derived from *Gallus gallus* Mx protein mRNA. Accession number of each sequence is depicted in the tree.

4. Discussion

Chickens are natural hosts to influenza virus (Easterday, 1975) and many other viruses, and virus infection causes serious illness or death in chickens. The avian influenza virus (AIV) is also infectious to humans and has led to an acute condition in some cases (Subbarao *et al.,* 1998). Selective breeding of AIV-resistant chickens would be beneficial for both the livestock industry and human health. Therefore present study was conducted to determine the avian influenza resistant gene or *Myxovirus* resistant gene (Mx) and its diversity in chicken and duck. For this purpose blood samples from six groups of chicken and four groups of duck were analyzed by PCR and PCR-RFLP. In this study two different sets of primers were used to detect *Mx* gene and its diversity in the sampled population. These primers are reported by Sironi *et al.,* (2010) and Seyama *et al.,* (2006). We used primer set reported by Sironi *et al.,*(2010) to amplify ~299bp fragment only for detection of *Mx* gene. On the other hand *Mx* gene detection and diversity analysis was done by PCR-RFLP with mismatched primers and restriction enzymes reported by Seyama *et al.,* (2006). We could amplify the specific sized DNA using these reported primers (Fig. 1 and 2). However, the detection rate varies with the different sets of primers (Table 1 and 2). The overall *Mx* gene detection rate was higher (75.7%) with the primer reported by Seyama*et al.,* (2006) than the primer reported by Sironi *et al.,* (2010)

(43.6%) (Table 1 and 2). These variations might be due to different primers because the primers were designed based on different location of *Mx* gene with different targets. It is reported that Sironi-F primer anneals to the last intron of the *Mx* gene and *Mx*-Sironi-R primer anneals to the last axon on the gene (Sironi *et al.,* 2010) while NE2-F2 bind to axon 14 of the *Mx* gene Seyama *et al.,* (2006). Samples with negative result were tested at least three times to minimize error to confirm the true negativity. From these findings it seems primer reported by Seyama *et al.,* (2006) would be more suitable to determine the *Mx* gene in chicken and duck.

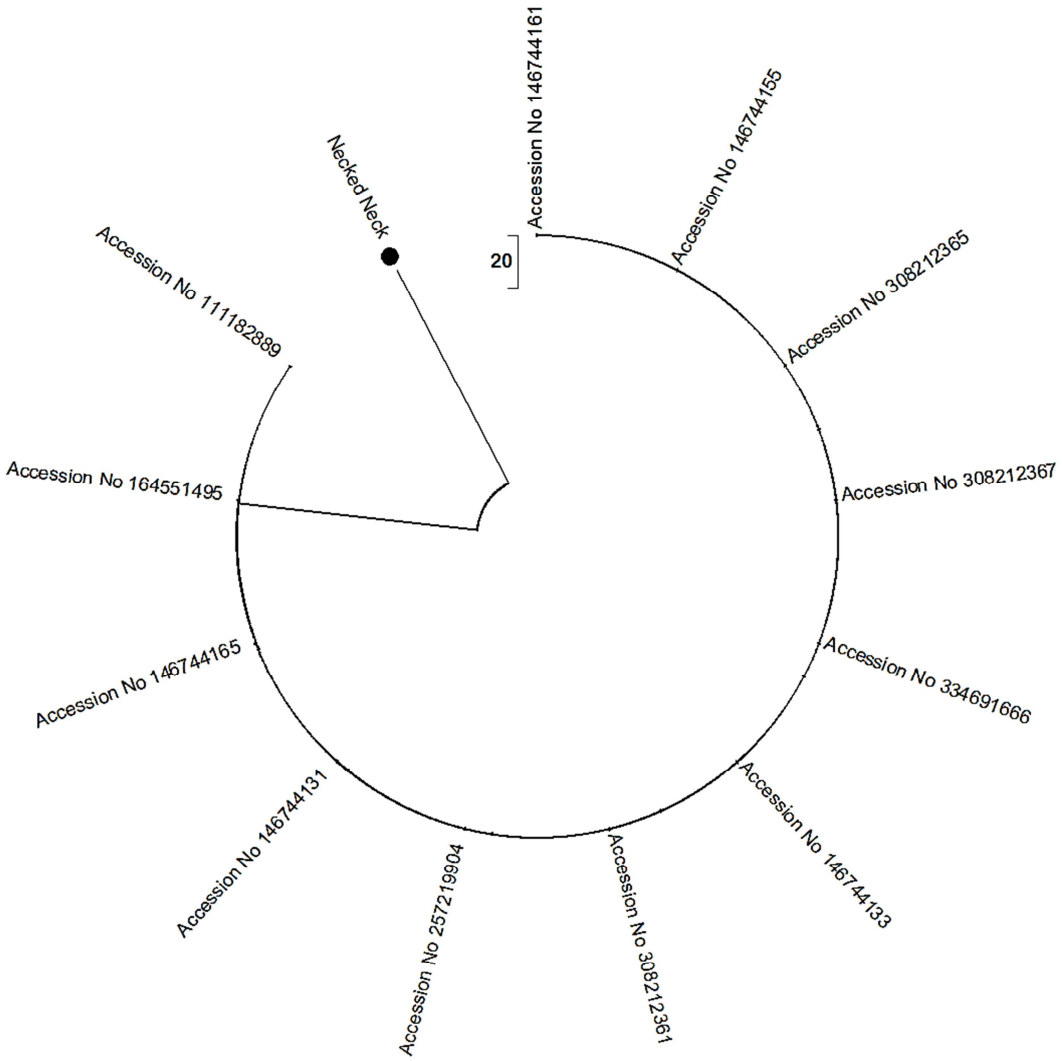

Fig. 3. Phylogenetic analysis of portion (98bp) of *Mx* gene of indigenous chicken necked neck. An unrooted neighbor joining tree of nucleotide sequences of *Mx* gene were generated. Sequences right and left side of the midline has 95 and 93% homology, respectively with the sequence of the *Mx* gene of necked neck (marked in the three with black circle) in the middle of the tree.

Mx protein induced by type I IFN is known to inhibit the multiplication of various viruses, including influenza virus (Lee and Vidal, 2002). However, chicken Mx protein in German White Leghorn lacked antiviral activity for both influenza and vesicular stomatitis viruses (VSV) (Bernasconi *et al.,* 1995). Similarly, Benfield *et al.,* (2008) reported that breed Shamo, which has an Asparagine at amino acid 631 is void of activity against the H5N1. These variations are due to variation in *Mx* gene. Because it is reported that a specific amino acid substitution between Asn and Ser at position 631 determines the differential antiviral activity of chicken Mx protein (Ko *et al.,* 2002). In the present study we determine the diversity of *Mx* gene and we found diversity is existed in the tested samples. Three types of results R/R, R/S and S/S were found. Diversity of *Mx* gene not only observed among the groups but also within the groups (Table 3). Indigenous chicken (Hilly and Necked neck) were found either resistant or heterozygous. We have tested only six samples and none was grouped into sensitive type. Our findings comply with the findings of Seyama *et al*. (2006). They investigated 271 DNA of commercial and indigenous chickens from different sources and found resistant, sensitive and heterozygous *Mx* gene allele within and between the groups of chicken. Similar results were

also reported by Sartika *et al*. (2011), Sulandari *et al*. (2009). They also reported that indigenous chicken has more frequency of resistant type *Mx* gene allele. Findings of the present study regarding distribution of *Mx* gene have likeness with the findings of Seyama *et al.*, (2006). We found 29.6, 44.4 and 25.9% of the tested sample asresistant, sensitive and heterozygous *Mx*gene alleleic, respectively. While Seyama *et al*. (2006) reported 33.95% resistant, 52.40% sensitive and 13.65% heterozygous allele in the tested samples (n=271). Present finding also has likeliness with the findings of our previous work where we found 42.86% homozygous resistant (R/R), 42.86% homozygous sensitive (S/S) and 14.29% heterozygous.

Outbreak of HPAI was first occurred in Bangladesh in 2007 and since then it is continued to occur across the country. The disease is mostly occurred in commercial chicken (n=499) than native chicken (n=57) (Giasuddin *et al.,* 2013). Though duck are considered to be resistant, an HPAI outbreak in native ducks in Netrokona district was reported in 2011 (ICDDR,b, 2013). However, no such occurrences of HPAI were reported from rest part of the country. But in Indonesia ducks are appeared to be mostly affected species with 25-50% mortality in juvenile, 19% in backyard and 33% in commercial duck (FAO, 2014). These findings suggest there may be some host factor responsible for lower occurrences of HPAI in native poultry and duck population in Bangladesh. Presence of *Mx* gene in the chicken and duck population may be that host factor. We have examined only 9 duck samples for *Mx* gene diversity analysis in this study and found that 66.6% samples were belonging to sensitive group while only 11.1% belong to resistant type and rest 22.2 heterozygous. This finding contradicts with the report of lower number of HPAI occurrences in our duck. Because if ducks are more sensitive then more outbreak should be occurred in this group and eventually reported number should be more in duck. However, our findings comply with the notion that ducks are reservoir of AIV. For harbouring and maintenance virus should undergo replication cycle in the host cell. But if the duck's cytoplasmic Mx protein inhibits the replication then AIV could not maintain in duck.

5. Conclusion

The *Mx* gene is amplified from all the six groups (two indigenous & four exotic breeds) of chicken and four groups of duck. Three types of *Mx* gene allele are found in the study. These are homogenous resistant ((R/R), homogenous sensitive (S/S) and heterozygous (R/S). Diversity is found within and between groups of chicken and duck. Sequence analysis of portion of *Mx* gene of necked neck indigenous chicken showed 95% homology with *Mx* gene from White Leghorn and other chicken *Mx*gene.

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