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Bottleneck Effect Analysis in Indigenous Poonchi Chicken Located Near International Border of India and Pakistan

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ABSTRACT

Background: Poonch is the remotest district of Jammu, India located near to line of control with Pakistan. Poonchi chicken is the first native poultry breed of Jammu region which is being characterized and registered with National Bureau of Animal Genetic Resources. Present study is the first study on genetic bottleneck assessment in Poonchi chicken population using recommended microsatellite markers. This study in Poonchi chicken would be of immense use in tracing evolutionary origin and formulating effective conservation strategies for genetic diversity within breeds.

Methods: Blood samples were collected randomly from Poonch and Rajouri districts of Jammu for DNA extraction and analysis. Microsatellite markers selected for the analysis were ADL0268, MCW0206, MCW0081, ADL0278, MCW0069, MCW0248, MCW0111, MCW0222, MCW0016 and LEI0094. To evaluate the Poonchi chicken for mutation drift equilibrium, Bottleneck software was used. Infinite allele model (IAM), two phase model of mutation (TPM) and stepwise mutation model (SMM) tests were done. Mode shift test was also used as another method of qualitative test for allele frequency.

Result: Total number of alleles observed were 72, with the total number alleles ranged from 6 to 9. The results of all three models (IAM, TPM, SMM) in Sign test depicted the heterozygosity was more in the expected number of loci in Poonchi chicken as 5.97 ($P<0.05$), 5.93 ($P<0.05$), 5.90 ($P<0.05$) respectively. Whereas for Wilcoxon rank test the IAM, TPM and SMM revealed significant values of ($P<0.05$) deviation indicating that all the loci deviate from mutation-drift equilibrium. Mode shift test graph obtained depicted a slight shift from normal L-shaped curve indicating recent bottleneck effect in Poonchi chicken. This might have resulted in the loss of number of effective alleles which suggests a need of planning and implementing an effective breeding policy in order to preserve and promote this native breed.

Key words: Genetic bottleneck, Infinite allele model (IAM), Microsatellite, Poonchi chicken, Two phase model of mutation (TPM) and Stepwise mutation model (SMM).

INTRODUCTION

Livestock production constitutes a very important component of the agricultural economy of developing countries, with multipurpose uses, such as skins, meat, egg, fibre and fertilizer (George *et al.,* 2024). Poonch and Rajouri (Fig 1) are remote districts of Jammu located near line of control and share international borders with Pakistan. Backyard poultry farming with local native indigenous chicken is common practice in these hilly areas. These native indigenous chickens play an important role in socioeconomic upliftment of hilly tribal farmers. The present study was carried out with the main aim to evaluate the presence or absence of bottleneck effect in the Poonchi chicken as previously no study has been done in Poonchi chicken using these recommended microsatellite markers. Earlier studies have documented that local indigenous breeds /strains exhibit more diversity as compared to exotic commercial breeds (Lee *et al*., 2000; Okumura *et al*., 2006). It is very imperative to chracterize these native breeds and register them with national breed registration organization for conservation and research purposes. Breed registration involves genotypic chracterization using different molecular markers for estimation of genetic variabilty. Microsatellite markers have been reported by many research workers as one of the most accurate and easy ways to estimate genetic diversity estimation and its relationships among populations (Takezaki and Nei, 1996). Microsatellite

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markers are highly polymorphic, randomly repeated motifs or simple sequence nucleotide repeats (Tautz, 1989).

The term ″bottleneck″ refers to narrow neck of a bottle which decreases the flow of any substance into the bottle. In conservation studies population bottlenecks attributes to the loss of genetic variation in the population in due course of time due to drastic climate change, predation, limited resources that lead to random fluctuation in the size of the existing population. This reduction in size of the populations results in development of smaller gene pool, this decrease in gene pool is not contemplative of the diversity in the original gene pool. Probable reasons for genetic bottle neck in Poonchi chicken population is loss of genetic diversity due to natural disaster like flash floods in hilly areas of Jammu and Kashmir, inbreeding and small population size of Poonchi chicken flocks. Founder effect is another reason for reduction in diversity as a large habitat of the Poonchi chicken have gone to Pakistan after independence. Microsatellite markers have been used for bottleneck analysis in various livestock species like cattle (Ganapathi *et al*., 2012; Thiagarajan, 2012), sheep (Girish *et al*., 2007; Radha *et al*., 2011) goat (Thiruvenkadan *et al*., 2014) and poultry (Vij *et al*., 2006; Pandey *et al*., 2005, Sharma *et al*., 2017. Microsatellite markers are useful in assessing genetic diversity, population structure and gene flow analysis and identifying inbreeding and out breeding levels. Microsatellite profiling helps assess the genetic variability within and between population, helps to identify the low genetically diverse population and simultaneously helps to conservation actions. Microsatellite markers can detect levels of inbreeding within a population, guiding breeding plans to introduce new genetic material or promote outbreeding to enhance heterozygosity.

MATERIALS AND METHODS

Blood samples were collected aseptically from wing vein of Poonchi chicken from remote villages of Poonch (Gursai, Ari, Harni, Digvaar) and Rajouri district (Androoth, Hanjana, Guni, Rajpur Bhatta) Rajouri. Genomic DNA isolation was carried out by both phenol-chloroform method and by sigma AldrichGenelute blood genomic DNA kit.50 best DNA samples with spectrophotometer values ranging from 1.7 to 1.9 were selected for further PCR amplification with FAO recommended microsatellite markers namely ADL0268, MCW0206, MCW0081, ADL0278, MCW0069, MCW0248, MCW0111, MCW0222, MCW0016 and LEI0094 (Table 1). PCR was standardized for all 10 primers and positively amplified samples were then resolved at 10% urea polyacrylamide gel electrophoresis for a period of 4hrs at 100 -120 volts. The gels were then stained with silver staining to visualize and analyse the number of alleles in each sample.The bands obtained after staining were further genotyped, scored and different alleles were identified as different bands. Alleles were marked as A,B,C..... in ascending order of size. Popgen 32 software was used to analyze the genetic diversity. Three tests Infinite allele model (IAM), two phase model (TPM) and step wise mutation model (SMM) were performed to evaluate the Poonchi chicken for mutation drift equilibrium. Three tests namely sign rank, standardized differences and wilcoxon test were

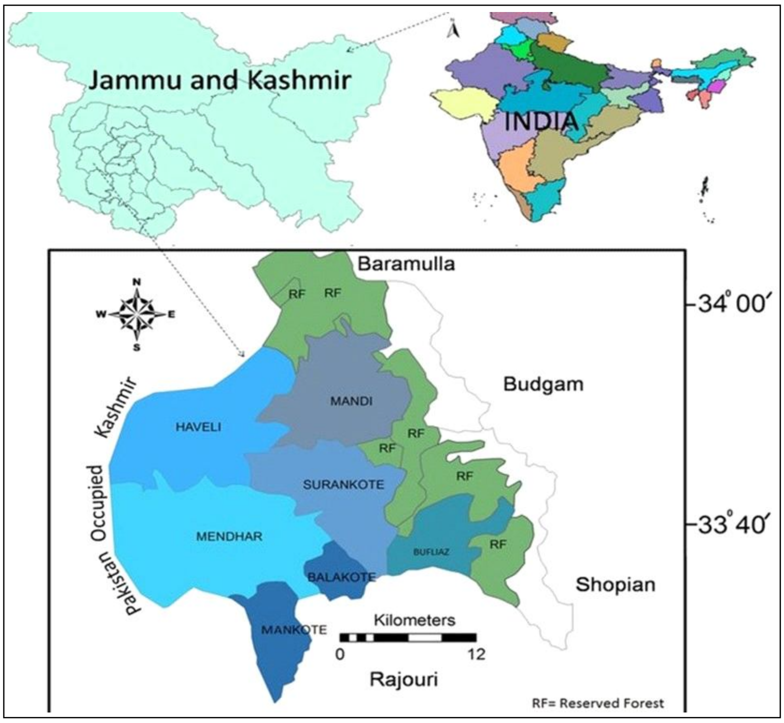

Fig 1: Various natural breeding tract of Poonchi chicken mainly Poonch and Rajouri district (Jammu and Kashmir).

used to investigate the presence of bottleneck effect in Poonchi chicken breed. The method for detecting bottleneck effect involves comparison of expected heterozygosity(H_e) with heterozygosity expected at mutation disequilibrium (H_{eq}).If H_e is higher than H_{eq}then there is heterozygosity excess whichmeans a recent bottleneck effect (Cornuetand Luikart 1996). Heterozygosity excess was tested with one tailed wilcoxon sign rank using bottleneck software 1.2.02 (Piry *et al.,*1999).The allele frequency distribution was established to see absence or presence of bottleneck effect. For qualitative graphical representation of allele frequency distribution another test, mode shift graph (Luikart *et al*.,1998) was used to identify potential bottlenecks. With the use of Mode shift graph allele frequency classes of ten microsatellite markers was obtained. L-shaped distribution curve of the allele frequency graph indicates no recent bottleneck effect and a shift from L-shaped distribution is suggestive of a recent genetic bottleneck.

RESULTS AND DISCUSSION

Chicken represents a valuable genetic resource and diversification of the poultry production is one of the viable options to enhance the food production (Kaushik *et al.,* 2023). Poonchi chicken is an indigenous breed reared mainly in remote areas of Rajouri and Poonch, Jammu and Kashmir (UT). Severe bottlenecks and population decline often lead to the reduction of most important factor *i.e.* genetic variation in a population (Mundy *et al*. 1997; Groombridge *et al.,* 2000). Documentation have been done by researchers to acknowledge the importance of variation for survival (McAlpine 1993; Keller *et al.,* 1994), the clutch size (McAlpine 1993) and growth rate (Alvarez *et al.,* 1989). In contrast to it some researchers observed no or little effect of these parameters. (Ardern and Lambert 1997). However, like many other indigenous breeds, Poonchi chicken is facing threats from the widespread use of commercial breeds. Since very less information regarding this indigenous population is available, it is very important to characterize, promote and conserve this breed. Therefore, the present study was carried out for bottleneck analysis of Poonchi chicken using microsatellite markers.

Bottleneck occurs when populations experience severe temporary reduction in size (Barani *et al.,* 2024). Bottleneck hypothesis was investigated using bottleneck software 1.2.02. The result for Bottleneck analysis under three microsatellite evolution models are presented in Table 2. Result showed that the number of alleles in the present study ranged from 6 to 9 for recommended microsatellite markers under study. It can be seen that the expected heterozygosity ranged between 0.73 (LEI0094) to 0.859 (MCW0111). Three tests IAM, TPM and SMM were performed to evaluate the Poonchi chicken for mutation drift equilibrium. All three models in Sign test showed expected numbers of loci with heterozygosity excess in Poonchi chicken as 5.97 ($P<0.05$), 5.93 ($P<0.05$), 5.90 ($P<0.05$) respectively. Whereas for Wilcoxon rank test the IAM, TPM and SMM revealed significant values of ($P<0.05$) deviation indicating that all the loci deviate from mutation-drift

Table 1: Primer sequence of different microsatellites used in the study.

Chromosome	Markers	Primer sequence (5′-3′)	Product size (bp)	Annealing temperature
1	ADL0268	CTCCACCCCTCTCAGAACTA CAACTTCCCATCTACCTACT	102-116	53°C
8	MCW0206	CTTGACAGTGATGCATTAAATG ACATCTAGAATTGACTGTTCAC	221-249	60°C
2	MCW0081	GTTGCTGAGAGCCTGGTGCAG CCTGTATGTGGAATTACTTCTC	112-135	59°C
5	ADL0278	CCAGCAGTCTACCTTCCTAT TGTCATCCAAGAACAGTGTG	114-126	60°C
E60C04W23	MCW0069	GCACTCGAGAAAACTTCCTGCG ATTGCTTCAGCAAGCATGGGAGGA	158-176	55°C
1	MCW0248	GTTGTTCAAAAGAAGATGCATG TTGCATTAACTGGGCACTTTC	205-225	58°C
1	MCW0111	GCTCCATGTGAAGTGGTTTA ATGTCCACTTGTCAATGATG	96-120	56°C
3	MCW0222	GCAGTTACATTGAAATGATTCC TTCTCAAAACACCTAGAAGAC	220-226	54°C
3	MCW0016	ATGGCGCAGAAGGCAAAGCGATAT GGCTTCTGAAGCAGTTGCTATGG	162-206	55°C
4	LEI0094	GATCTCACCAGTATGAGCTGC TCTCACACTGTAACACAGTGC	247-287	54°C

equilibrium (Table.3). Result show that the observed heterozygosity excess (He) is more than expected heterozygosity excess (Hee). The higher values of He then Hee indicate the presence of recent bottleneck effect in the Poonchi chicken. Probable reasons for genetic bottleneck in Poonchi chicken population are natural calamities/flood (that occurs during the 2014 causing huge loss in genetic resources and population size drastically reduced which leads to loss of genetic diversity), inbreeding and small population size (Poonchi chicken is reared in small flocks and there is no systematic breeding knowledge among farmers, inbreeding occurs in the population causing reduction of genetic diversity over time and increasing the likelihood of genetic bottlenecks) and geographical relocation (Poonchi bird is unique in nature and after independence some part of natural habitat goes to Pakistan, that left with a small group of individuals which subsequently establishes a new population; hence the genetic variation is limited to Poonchi chicken leading to a genetic bottleneck). Similar results were observed by De la Para *et al*. (2024) in light Brahma breed.

Bottleneck effect in Poonchi chicken can be improved or removed by introduction of genetically diverse Poonchi chicken population by the farmers or exchange of breeding birds among themselves. Farmers should be trained for scientific breeding management among Poonchi chicken to control inbreeding.

Mode-shift test

Mode shift test was used as another method of qualitative test for allele frequency. Luikart *et al.* (1998) proposed mode shift test for qualitative graphical representation of allele frequency distribution. The graph obtained depicted a slight shift from normal L-shaped curve indicating recent bottleneck effect in Poonchi chicken. (Fig 2). A normal L shaped distribution of the allele frequency graph is an indication of no recent bottleneck. When there is some shift or change in L-shaped distribution of the studied populations it indicates recent genetic bottleneck. The result suggested that the Poonchi chicken population had undergone an effective recent genetic bottleneck (last 15-20 generations). The main reason for genetic bottleneck is indiscriminate and unplanned crossbreeding which has led to the loss of effective alleles due to small size population, inbreeding or mating which occur among relatives and consequent to it causes genetic drift. Similar to these results were reported by De la Para *et al.,* 2024 in various Chilean Chicken breeds namely Kollonka, Ketro, Trintre, Cogotepelado, Light Brahma and Barred Plymouth Rock. Comparative to this a normal L-shaped curve was observed by De la Para *et al*. (2024) in Kollonca de aretes breed, in Punjab Brown chicken by Vij *et al.* (2006), in Ankleshwar poultry population of India by Pandey *et al.* (2005),in Kaunayen chicken of Manipur by Sharma *et al*. (2017) indicating that these breeds have not experienced any recent genetic bottleneck.

Table 2: Bottleneck analysis under three microsatellite evolution models in Poonchi Chicken.

Microsatellite	Observed	He	I.A.M.				T.P.M.				S.M.M.			
locus	alleles (k_o)		Heq	S.D.	DH/sd	P	Heq	S.D.	DH/sd	P	Heq	S.D.	DH/sd	P
ADL0268	6	0.767	0.576	0.149	1.289	0.0440	0.655	0.105	10.80	0.0880	0.729	0.066	0.573	0.3310
MCW0206	7	0.829	0.623	0.131	1.568	0.0030	0.702	0.091	1.392	0.0130	0.771	0.052	1.128	0.0760
MCW0081	8	0.857	0.666	0.121	1.582	0.0000	0.738	0.076	1.569	0.0030	0.801	0.043	1.312	0.0370
ADL0278	6	0.803	0.570	0.150	1.550	0.0000	0.652	0.109	1.375	0.0130	0.727	0.067	1.139	0.0720
MCW0069	9	0.842	0.707	0.103	1.307	0.0180	0.769	0.070	1.045	0.0800	0.824	0.038	0.481	0.3790
MCW0248	7	0.798	0.620	0.140	1.271	0.0330	0.709	0.082	1.087	0.0980	0.767	0.058	0.549	0.3370
MCW0111	8	0.859	0.663	0.124	1.576	0.0010	0.744	0.077	1.500	0.0010	0.798	0.046	1.330	0.0260
MCW0222	6	0.822	0.575	0.148	1.665	0.0030	0.659	0.106	1.534	0.0030	0.727	0.067	1.413	0.0080
MCW0016	9	0.829	0.703	0.112	1.124	0.0590	0.776	0.062	0.857	0.1810	0.822	0.038	0.183	0.5010
LEI0094	6	0.703	0.566	0.156	0.879	0.2010	0.649	0.109	0.496	0.3670	0.726	0.066	-0.348	0.2780

Table 3: Test for null hypothesis under three microsatellite evolution models for bottleneck analysis in Poonchi Chicken.

Mutation Models	Sign test			Standardized differences test		Wilcoxon test: One tail for H deficiency	Wilcoxon test: One tail for H excess	Wilcoxon test: Two tails for H excess or deficiency
	Hee	He	P	T2	P	P	P	P
IAM	5.97	10	0.00575	4.368	0.00001	1.00000	0.00049	0.00098
TPM	5.93	10	0.00540	3.774	0.00008	1.00000	0.00049	0.00098
SMM	5.90	10	0.04064	2.454	0.00706	0.99902	0.00146	0.00293

Abbreviations and symbols:Hee: expected number of loci with heterozygosity excess; He: observed number of loci with heterozygosity excess; T2: test 2; P:probability value for heterozygosity excess; IAM: infinite allele model; TPM: two-phase model; SMM: stepwise mutation model;

*P<0.05 showing significant differences between the observed and expected values for heterozygosity excess.

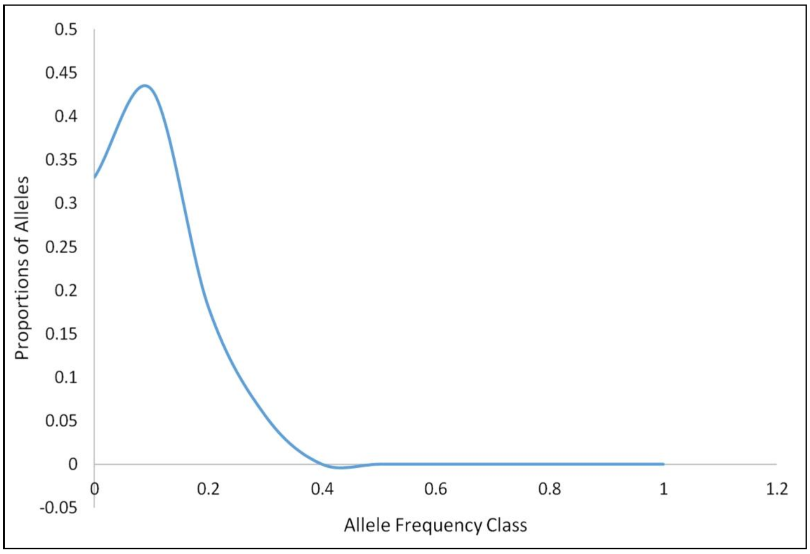

Fig 2: Mode shift test for bottleneck in poonchi chicken.

CONCLUSION

The presence of genetic bottleneck calls for an urgent need of implementing an effective well planned breeding policy to preserve and promote this native breed which is well suited for local conditions of Jammu and Kashmir. Genetic heterozygosity and inbreeding are indeed inversely proportional, meaning that as inbreeding increases within a population, genetic heterozygosity decreases. Although, the overall heterozygosity deficiency in the population is negative but the value is very close to zero which indicates loss of substantial heterozygosity in the population. This work is the first study done on Poonchi chicken using specific microsatellite markers indicating genetic bottleneck effect.

Conflicts of interest

There is no conflict of interest for authors to declare.

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